Development of a Quantification Method to Specific Anti-NS3 Antibody Against Bovine Viral Diarrhea Virus (BVDV) Using a Blocking ELISA

S. Guillossou[1], *DVM, MS;* **D. Thomson**[1], *PhD, DVM;* **C. Thomson**[2], *MS*
[1]*Kansas State University, Department of Clinical Science, Manhattan, KS*
[2]*Synbiotics Corporation, Manhattan, KS*

Introduction

There are numerous published epidemiological models in Europe concerning the control of the bovine viral diarrhea virus (BVDV) in cattle by identifying and removing persistently infected (PI) cattle from herds. It is difficult to adapt these models in countries that routinely vaccinate cattle for BVDV. Vaccination interferes with previous models when measuring total or serum neutralizing antibodies. An alternative approach could be to measure a subpopulation such as antibody targeting nonstructural proteins that can be found only during virus replication. The objective of the study was to develop a quantitative serum antibody test for BVDV with a commercially available test (SERELISA® BVD Ab Mono Blocking, Synbiotics Corporation) that is based on a blocking ELISA which allows specific detection of anti-NS3 antibodies against the BVDV. The blocking format alone does not allow a straightforward approach for developing quantification methods because of the usual lack of linearity of these tests. Therefore, statistical applications were explored to develop a quantitative extension of the blocking format.

Materials and Methods

Results of the test are expressed as sample to negative control optical density (OD) ratio corrected by the positive control OD and referred as s/n ratio. The linear range of the bELISA was determined by conducting the assay with the s/n ratio of a positive reference sample at different dilutions at 1:10, 1:50, 1:100, 1:200, 1:400, 1:500, 1:800, 1:1,000, 1:5,000 and 1:10,000, in order to recreate a panel of samples ranging from strong positive to weak positive. After graphical analysis, determination coefficients (r2) were calculated for eight different models with variable transformations for s/n ratio and the dilution of titer. Transformations were analyzed for the relationship between titer (T), 1/T, and log T and s/n ratio (sn), 1/sn, log sn and logit sn. Determinations of coefficients and regression equations were calculated using R version 2.4.1.

Results

Linear s/n ratio values ranged from 0.13 to 0.90 using the reference BVDV positive serum sample at different dilutions. Comparing eight different regression models correlating different s/n ratio and titer functions, the best model was achieved utilizing the Log of titer and the logit of s/n ratio. This model was linear with an r2 of 0.983, a slope of β=-0.737 and an intersection of α=1.661. This linear model covered at least a range of a log (base 10) of titer. Interpolation using different wells was needed to have a quantitative method valid for serum ranging from negative status to high positive titer. The s/n ratios exhibited linearity for titer ranging 1:10 to 1:200. An arbitrary decision was made to apply this result to the 1:100 dilution in the well. Derived from this decision, interpolation was calculated for the dilutions of 1:10 and 1:1000. The final model was not limited on the lower bound of the 1:10 well and an arbitrary limit was fixed on the upper bound of the 1:1000 well. Titers obtained were expressed in ELISA units (EU). Robustness observed on the selected model resulted in a coefficient of variation of 16.9% for the slope and 3.9% for the intercept on 8 repeated measures.

Significance

This study resulted in an innovative method that provides a quantitative model for the detection of specific anti-NS3 antibody. This linear and robust method is independent of the seroneutralizing properties of the targeted antibody and therefore could be an alternative to seroneutralizing tests. This standardized quantitative test is a tool that could lead to a breakthrough in the understanding of the subpopulation of antibodies against nonstructural proteins. This may allow epidemiologists to adapt models for BVDV control even when cattle are vaccinated.

ISSN 0524-1685

The Bovine PRACTITIONER

Guidelines for Authors

Two issues of *The Bovine Practitioner* are published annually, one in the spring and one in the summer. It also serves as a communication medium between bovine practitioner organizations around the world. All manuscripts and communications must be presented in English.

Most articles in the journal are peer-reviewed or refereed. Papers submitted for publication in the peer-reviewed section are anonymously reviewed by three members of the editorial board. In some cases, papers may be reviewed by an outside expert(s) who is not a regular member of the editorial board. Papers published in the peer-reviewed section of the journal will be identified with a "Peer-Reviewed" banner at the top of the first page. Papers rejected by the editorial board for publication as peer-reviewed articles do not automatically qualify for publication in the non-peer-reviewed sections.

Articles published in *The Bovine Practitioner* are intended to address the needs of bovine practitioners. Types of articles considered appropriate for the journal include research reports, case reports, review articles, retrospective studies and articles describing new techniques.

All papers should begin with an abstract. Research reports should follow with an introduction, materials and methods (including experimental design and statistical analysis), results, discussion and conclusions. At the author's discretion, results and discussion may be combined.

Case reports should be written to include an introduction, history, clinical findings, appropriate laboratory data, surgical/therapeutic management, discussion and conclusions.

Review articles covering topics important to the practitioner are welcome. They should address more recent advances and bring the reader cutting edge information related to bovine practice or to beef or dairy production.

Papers reporting retrospective studies should include an introduction, clinical implications or objectives of the study, the methodology used to evaluate the data, a section that details the significance of the findings to the practitioner and conclusions.

Two manuscripts and a diskette or CD should be submitted to the editor through the mail or via a parcel delivery service. Manuscripts should be double-spaced, using 12-point Times type and 1-inch margins. Both lines and pages should be numbered. When possible Microsoft Word should be used.

Figures, tables and photographs are welcome. Figures should be numbered on the back: legends for figures should be submitted on a separate sheet of paper. Photographs can be submitted as digital images or prints; prints are preferred over 2x2 slides.

English units of measure should be used for weights, measures and temperature. If the author desires, it is acceptable to follow English units with metric units in parenthesis, i.e....440 lb (200 kg) steer had a rectal temperature of 101.5°F (38.6°C). When the use of brand names is necessary, they should be listed in footnotes or endnotes, including the name of product, manufacturer, and manufacturer's city and state.

References to literature cited in the paper must be identified in the text by the use of superscripts. References should be listed in **alphabetical order**. Suggested style for citations in the reference section is as follows:

1. Allen WM, Sansom BF: Parturient paresis (milk fever) and hypocalcemia (cows, ewes, and goats), in Howard JL (ed): *Current Veterinary Therapy III. Food Animal Practice.* Philadelphia, WB Saunders Co, 1993, pp 304-308.
2. Barth AD, Cates WF, Harland RJ: The effect of body fat and loss of fat on breeding soundness classification of beef bulls. *Can Vet J* 36:758-764, 1995.
3. Nutrient Requirements of Beef Cattle, ed 7. Washington DC, National Academy Press, 1996.
4. Syvrud R: Vaccination for bovine respiratory syncytial virus: Benefits for both cow/calf and feedlot cattle. *Proc Am Assoc Bov Pract* 21:204-206, 1989.

All correspondence and manuscripts should be addressed to:

The Bovine Practitioner
Dr. Bob Smith, Editor
3404 Live Oak Lane
Stillwater, OK 74075
405-372-8666 Office
405-743-8422 Fax

Rev 04/07