Testing of Cattle Ear Notch Samples Using a Bovine Viral Diarrhea Virus NS3 Antigen ELISA Kit for Detection of Persistently Infected Animals

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Introduction

Identification and elimination of persistently infected (PI) cattle is important to controlling bovine viral diarrhea virus (BVDV) in herds. Compared to many diagnostic laboratory techniques, ELISA (enzyme linked immunosorbent assay) is convenient due to the ability to test large number of samples rapidly and consistently. A virus can be detected by probing for a specific nonstructural antigen like the NS3 (p80 in former nomenclature) or a structural antigen like the glycoprotein Erns (E0 or gp48 in former nomenclatures). Ear notch samples can be obtained easily and quickly from cattle. Another important advantage of ear notch skin biopsy samples is that they contain a high concentration of BVDV antigen. Few publications can be found describing the detection of NS3 in ear notches from cattle. The objective of this study was to compare the performance of a current commercial ELISA which specifically detects the NS3 antigen on ear notches from cattle to other tests utilized in diagnostic laboratories.

Materials and Methods

The expected prevalence of PI cattle is estimated between 0.2 and 2%. The limiting factor for a reference population of known PI and non-PI cattle is therefore the recruitment of these PI cattle. Positive samples used in this study were obtained from samples submitted and confirmed positive at the veterinary diagnostic laboratory (VDL) of Kansas State University (KSU). Negative samples were collected from the field. All samples were tested with at least the immunohistochemistry (IHC) or the Erns antigen ELISA (IDEXX®) used routinely at KSU VDL. Ear notch samples from 245 PI positive and 501 PI negative cattle were tested with the specific NS3 Ag ELISA (SERELISA® BVD p80 Ag Mono Indirect, SYNBIOTICS® Europe, Lyon, France). The NS3 Ag ELISA test was used following directions in the kit insert except for the sample preparation and the results expression sections. Ear notches were stored frozen (-20 C) in PBS. Then 100ul of PBS solution from the ear notch samples were added to the wells for the sample incubation. Optical densities were interpreted as sample to positive ratio corrected by the negative control. The performances of the test were assessed using a ROC curve analysis. After proposing a cutoff, sensitivity and specificity estimates were calculated.

Results

The SP range for the 501 negative samples was -0.06 to 0.60 with a mean of 0.06. The 245 positive samples ranged from 0.33 to 3.04 with a mean of 2.19. The positive population exhibited a tail in its distribution. The ROC curve analysis provides a significant separation between the populations with several possible positions for the cut off with optimum performances. The elected cut off of 0.50 leads to an observed sensitivity of 98.8% (95CI=[96.5%-99.6%]) and an observed specificity of 99.8% (95CI=[98.9%-100%]). This cutoff was located at 6.8 standard deviations of the mean of the negative population and at 3.6 standard deviations of the positive population.

Significance

An excellent agreement was observed between ear notch NS3 Ag ELISA test results and the reference methods used in this study. Test results demonstrated good sensitivity for this method despite the broad distribution observed with the positive population. The specificity observed was high and the distance between the negative population and the proposed cut off indicates a good positive predictive value. The positive predictive value is a key issue when using a diagnostic test in a population where the condition or disease is observed with a low to very low prevalence. The high positive predictive value was achieved by the positioning of the cut off and its distance to the negative population. This study demonstrates the suitability of a NS3 Ag ELISA for the detection of PI cattle in a population with low prevalence.