Effect of Varied Amounts of PBS and Time Before Application of PBS on the ACE for Detection of Calves Persistently Infected with Bovine Viral Diarrhea Virus

R.A Funk; D.U. Thomson, *DVM, PhD, MS;* **R.L Rust; B.J. White,** *DVM, MS;* **D.G. Renter,** *DVM, PhD*
Department of Clinical Sciences, Kansas State University, Manhattan, KS

Introduction

The identification and removal of persistently infected (PI) animals from a production system is a key component to controlling BVD. In order to achieve this, a reliable method of testing for PI animals must be used Ear notching is a common and easy way to obtain diagnostic samples in a relatively non-invasive manner. Previously these tissue samples were examined by immunohistochemistry for the presence of the BVD virus in the epithelium. Recently, advancements have been made that allows the testing of a phosphate buffered saline ear notch extract by enzyme linked immunosorbent assay (ELISA). This technique of detecting BVD infected animals is much more rapid than the conventional IHC testing. Additionally, ELISA testing methods do not require the level of specialized equipment or number of personnel that other methods such as ear-notch immunohistochemistry or polymerase chain reaction require, and readily adapt themselves to automation.

Materials and Methods

Objective 1: Determine the effect of varying amounts of PBS added to ear-notch samples on the sensitivity of antigen-capture ELISA. Ears were collected from nine known PI animals and 8 known PI-negative animals at necropsy. These ears were notched to yield 1 cm X 1cm tissue samples, and samples were placed in 0.1 M PBS for BVD testing. The amount of PBS added to samples was 1ml, 2ml, 4ml, 6ml, 8ml, and 10ml for each of the notches collected for each animal. Samples were allowed to sit in PBS for 48 hours; after which, all samples were subsequently tested for BVD status utilizing ACE. Objective 2: To determine the effect of delayed addition of PBS to ear-notch samples on the sensitivity of antigen-capture ELISA. Ears were collected from nine known PI animals and 8 known PI-negative animals at necropsy. Ears were notched in 1 cm by 1 cm samples, and tissue was placed in tubes to have 0.1 M PBS added at specific intervals. PBS was added to samples at 0, 1, 4, 8, 12, and 24 hours post-notching. All samples were subsequently tested for BVD status utilizing ACE.

Results

Increasing the amount of PBS added to a sample significantly decreased the mean S/P ratio determined by ACE testing ($P = <0.01$). Despite this, there is no significant difference in the ability of the test to detect positive individuals across all treatments ($p = 0.82$) even though at one treatment (6 ml) the detection level for BVD dropped below 100%. The delayed addition of PBS to ear notch samples did not affect the detection rate for BVD, which remained at 100% across all treatments. There were statistically significant differences in the S/P ratios at different treatment levels. The lowest S/P ratios occurred when PBS was added to the samples at 2, 4, or 8 hours after the notch was collected. The highest S/P ratios occurred when PBS was added immediately, 1 hour, 12 hours, or 24 hours post-sample collection.

Significance

There are times in veterinary practice that samples will get mishandled. Attempts were made to simulate the most common errors seen when practitioners call in about samples. First, diluting the sample by adding additional PBS at the time of sampling had a significant affect on the S/P ratios of the tests. There was not a significant decrease in S/P ratio until 6 ml of PBS was added to the ear notches. Although the S/P ratio decreased, additional amounts of PBS to the sample vials did not decrease the sensitivity of the test. There are times when practitioners forget to bring PBS to the field or find themselves on a call with no sample vials. The question arises as to how long can you wait until you get the sample placed in PBS and cooled in the refrigerator? This scenario was simulated in the lab by ranging time until PBS was added to the samples from no delay up to 24 hours. To a lesser degree, delayed time to addition of PBS while showing some affect, did not appear to have the affect on the ability to detect positive individuals up to 24 hours post-sample collection in the field.