

Parameters of Ear Notch Samples for BVDV Testing: Stability, Size Requirements and Viral Load

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Research Summary

Introduction

Infection with bovine viral diarrhea viruses (BVDV) is a major source of economic loss for the US cattle industry. Houe (2003) estimates that losses in areas where BVD is endemic range between \$10 to 40 million per million calvings. Results of serology surveys in the US suggests that our losses are in the upper end of this range (Houe *et al*, 1995a; Houe *et al*, 1995b; Paisley *et al*, 1996). In 2004, 37.6 million calves were born in the US; thus, US losses by this estimate would be between \$376 million to 1.5 billion for 2004. The 2005 calf crop was ~37.8 million, making the 2005 estimate very similar to that of 2004. While BVDV infections are well recognized as reproductive pathogens among dairy producers, producers are less aware that BVDV infections are also associated with increased respiratory disease, increased severity of secondary infections and decreased milk production. Persistently infected (PI) animals are the major vectors for spreading BVDV within and among herds. Based on studies of dairies of 100 cows or more, Joly *et al* (2005) estimated that the presence of one PI animal in a herd resulted in a loss of \$1.93/cwt of milk sold. Studies estimate that 10-15% of US herds have at least one PI animal (Houe *et al*, 1995c; Wittum *et al*, 2001). In 2004, US dairies produced 170,805 million lb (77,638 kg) of milk. If 10% to 15% of dairy herds have at least one PI animal, then the cost to milk production is between \$330 to 494 million per year. Control efforts in the US are geared towards identifying and eliminating PIs. Several tests based on detection of either antigen or viral RNA in blood, serum, bulk milk or skin biopsies are currently in use (Cornish *et al*, 2005; Fulton *et al*, 2006). Ear notch samples have become the tissue of choice for screening for PI animals because 1) they are easy to collect; 2) equipment requirements are minimal; 3) they are not affected by presence of passive antibodies; and 4) they can be used as the sample for a wide variety of tests including immunohistochemistry, real-time polymerase chain reaction and antigen-capture ELISA. While ear notches have become one of the samples of choice, there is little information available regarding sample size requirements and stability. Further, while pooling of ear notch samples has been proposed for reducing the cost of surveillance programs (Kennedy *et al*, 2006), the viral load available for detection from ear notch samples is largely undetermined. The purpose of this study was to establish working parameters for sample size, viral detection limit and sample storage conditions for real-time PCR and antigen-capture ELISA.

Materials and Methods

Real-time polymerase chain reaction (PCR) and antigen-capture ELISA (ACE)- based BVDV detection tests were used. The first series of experiments used fresh ear notches and evaluated the amount of sample required, effects of storage conditions and effects of dilution on detection methods. Fresh ear notch samples were extracted by soaking for 60 minutes in phosphate buffered saline (PBS). In the first set of experiments, ear notches were subdivided into weights ranging from 0.75 gm to 0.02 grams. In a second set of experiments, ear notch samples were stored at -4°F (-20°C), 39°F (-4 °C), room temperature 77°F (25°C), 98.6°F (37°C) and lyophilized. A third set of experiments focused on the extent that fresh ear notch extractions could be diluted before detection, dropped off. The second series of experiments were done to determine the range of virus concentrations found in ear notch extractions. These experiments were done on samples in which the ear notch had been frozen in PBS at -4°F (-20°C). Virus concentrations were determined by comparing test values against a standard curve constructed using titrated viral stocks.

Results

There was no difference in the amount of virus detected in ear notch extractions using ear notch samples weighing between 0.75 and 0.05 gms. However, samples weighing 0.03 gms or less resulted in extractions containing at least a log lower virus concentration. There were no significant differences between storage at -4°F, 39°F and 77°F for seven days. In contrast, detection was reduced in lyophilized samples and samples

stored at 98.6 ° F for seven days. The concentration of virus in ear notch extractions averaged 452.3 virions/ml. Of the 153 samples evaluated for virus concentration, 16 (10.5%) had between 10 and 100 virions/ml, 86 (56.2%) had between 100 and 1000 virions/ml, 50 (32.6%) had between 1000 and 10,000 virions/ml and one (0.7%) had more than 10,000 virions per ml. The detection limit of the real time PCR test was determined to be 10 virions/ml.

Significance

These results suggest that ear notch samples are relatively stable for at least seven days if stored between -4 ° and 77 ° F. However, exposure to higher temperature and drying both reduced detection. Similarly, the amount of virus extracted was not significantly affected by sample size over a wide weight range. The concentration range of virus in ear notch extractions and the detection limits of real-time PCR suggest that pooling of samples in surveillance programs must be approached cautiously. Pooling of 10 samples, where a sample pool includes one positive and nine negative samples, could result in the failure to detect 10% of the samples used in this study. Pooling of 100 samples, where sample pool includes one positive and 99 negative samples, could result in failure to detect over 50% of the samples used in this study.

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