Establishment of Presumptive Diagnostic Cut-Off for Persistently Infected Cattle

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ABSTRACT

Bovine Viral Diarrhea Virus causes infection in cattle that has led to major economic losses in both the beef and dairy industries. *In utero* BVDV infection can induce immunotolerance, causing animals to be persistently infected (PI) for life. PI animals continuously shed the virus and are the main source of BVDV infection in herds. Animals that are acutely or transiently infected will pass the disease and will show negative BVDV results if re-tested 2 weeks after an initial positive test. In this study, we show a method for presumptive PI determination based on real-time RT-PCR results.

Life Technologies's Bovine VetMAX™- Gold BVDV Detection Kit has been shown to be a rapid method for BVDV detection. Ear punch samples were used since ear punches will show high viral titers if an animal is PI, but not if the animal is acutely infected. Purified RNA from 63 BVDV PI positive samples of various subtypes and 53 BVDV negative samples co-mingled with PI animals to create transiently-infected (TI) cattle that were tested with the Bovine Virus Diarrhea RNA Test Kit. A 3mm ear punch re-suspended in 200µl PBS, using 50µl of supernatant for MagMAX Viral Isolation followed by real-time RT-PCR with BVD Detection Kit on the AB 7500 Fast will yield a Ct < 31 if the animal is a presumptive PI. Secondary testing is required to determine PI status. Once the presumptive diagnostic cutoff was made for beef cattle, a set of 29 confirmed PI ear notch samples from dairy cattle were tested with the Life Technologies' VetMAX™-Gold BVDV Detection Kit.

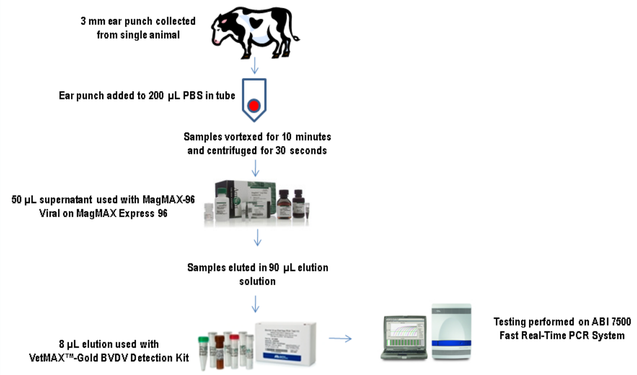
INTRODUCTION

The VetMAX™- Gold BVDV Detection Kit was the first USDA licensed kit launched by Life Technologies. Bovine Viral Diarrhea Virus (BVDV) causes infection in cattle that has led to major economic losses in both the beef and dairy industries estimated to be up to three billion dollars annually. *In utero* BVDV infection can induce immunotolerance of the fetus, causing animals to be born persistently infected (PI) (Peterhans et al. 2010). Rapid detection of PI cattle is essential for BVDV control. PI animals are routinely diagnosed by retesting samples 2-3 weeks after an initial positive result. Thus, the aim of this study was to determine if the Life Technologies' VetMAX™-Gold BVDV Detection Kit can be used to establish a suitable diagnostic cut-off for presumptive PI cattle for ear notch samples obtained from a mixed population of beef cattle.

MATERIALS AND METHODS

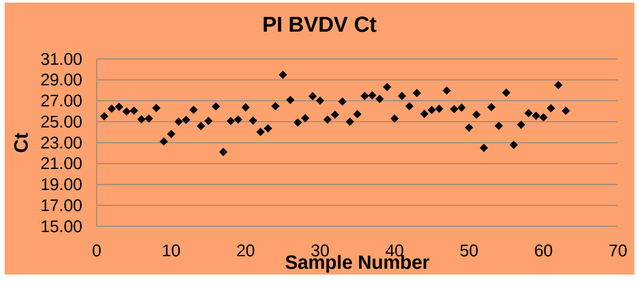
In this study, 3 mm ear punches were used. 63 animals were confirmed to be PI by testing positive by antigen capture ELISA twice over at least 3 weeks. The workflow for testing is outlined in Figure 1. From these 63 PI animals, 10 animals were selected that represented BVDV subtypes found in the field as shown in Table 1. These 10 PI animals were commingled with 53 BVDV negative animals to make TI animals. Ear punches were taken from these BVDV negative animals at arrival day, then days 8, 13, and 20 after commingling. Statistical analysis was performed at KSVDL by Stephane Guillossou. Ear notches (1cm x 1cm) from dairy cattle from 29 confirmed PI were resuspended in 2 ml of PBS, incubated for 1.5 hours, followed by vortexing. Fifty µl of the supernatant was used as input for MagMAX viral isolation.

Figure 1. BVDV Testing Workflow



RESULTS

Figure 2. PI BVDV Ct Results



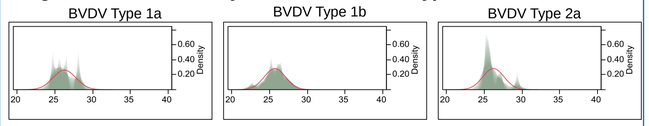
Scatter plot of Ct values observed from 63 confirmed PI animals by 2 positive tests separated by 3 weeks in time. A low Ct, corresponding to the highest titer, was observed at 22.22. A high Ct, corresponding to the lowest titer, was observed at 29.49

Table 1. Effect of BVDV Subtype on Ct

Subtype	South Midwestern US (Ridpath paper)	63 PI Study Population	10 PI Subset Population
1A	12%	6%	20%
1B	75%	78%	60%
2A	13%	16%	20%

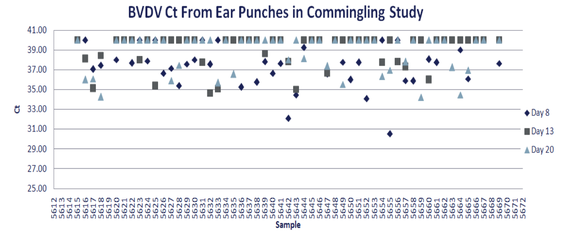
BVDV subtypes from PI study are representative of what is observed in the field

Figure 3. Kernel Density Plots of BVDV Subtypes



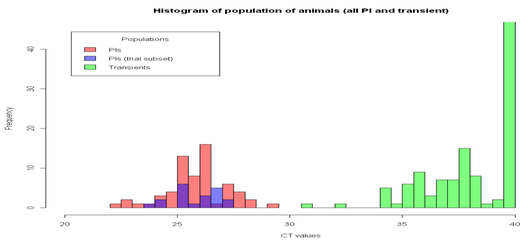
Density plots of all three subtypes centered around cycle 26
No statistical difference of Ct observed from different BVDV subtypes

Figure 4. TI Ear Punches Results



Ct values observed for the 53 BVDV negative animals commingles with PI animals. Ear punches collected on arrival day, days 8, 13, and 20 days after commingling with PI animals. Lowest Ct observed was 30.51

Figure 5. Combined PI and TI Data From Ear Punches



Combined histogram of all animals from study, PI and TI. Data shows 10 animals used as subset of PI population was representative of the PI population. Data also shows no overlap of Ct's from PI and TI populations between Ct 29.49 and 30.51.

Figure 6. Kernel Density Plots of BVDV PI Dairy Cattle

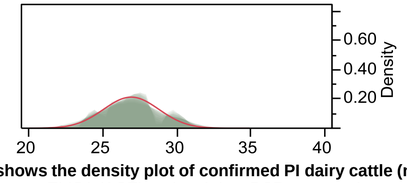


Figure 6 shows the density plot of confirmed PI dairy cattle (n=29) Ct > 31. Antigen capture ELISA (ACE) and real-time PCR was used to test 29 samples from positive dairy cattle. Whole blood and ear notches were re-submitted no earlier than 4 weeks apart after the original testing to determine PI status.

Table 2. Statistical Analysis of Presumptive Ct Cutoff

Day Post Infection	Ct		
	30	30.5	31
Se	99.32%	99.72%	99.89%
SP (day 8)	98.37%	97.82%	97.09%
SP (day 13)	98.89%	98.47%	97.89%
SP (day 20)	99.98%	99.95%	99.88%
SP transient	99.51%	99.27%	98.91%
SP neg	-	-	-

In a parametric approach, sensitivities and specificities calculated for presumptive PI Ct cutoffs at 30, 30.5, and 31 at a 95% tolerance limit. The following estimates would be found for a cutoff of 31 with a tolerance limit of 95% to the current population in feedlots. Sensitivity (PI) = 99.9% (Specificity transient) = 98.9% Specificity (negative-naïve) = 100%.

CONCLUSIONS

- 3mm ear punches can be used to differentiate Presumptive PI from TI positive samples
- Presumptive diagnostic cutoff for PI determination is established at Ct 31. Samples yielding Ct ≤31 are considered presumptive PI
- Samples from TI animals will mostly be negative or have Ct > 31 For final PI determination, a second test must be performed at least 3 weeks after an initial positive test to confirm PI status.
- A field study report, entitled "Establishment of Presumptive Diagnostic Cut-Off for Persistently Infected Cattle" was approved by USDA-CVB to support revised labeling for this product.
- **Presumptive PI/TI diagnostic claim for USDA license has been approved**
- Ear notch samples from confirmed PI dairy cattle showed Ct ≤31.
- Samples form dairy cattle were much closer to the upper limit Ct 31 which could be explained by sample age and degradation.
- A presumptive PI Cut-Off could potentially be the same for Dairy Cattle; further research is warranted.

REFERENCES

1.Peterhans, E., Bachofen, C., Stalder H., and M. Schweizer. 2010. Cytopathic bovine viral diarrhea viruses (BVDV): emergin pestiviruses doomed to extinction. Vet. Res. 41:44.

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