Ear Notch Extract PCR - a cost effective approach for screening feedlot cattle

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Diagnosis on the Ear Notch Substrate

- Immunohhistochemistry
  - Njaa. J Vet Diagn Invest. 2000 Sep;12(5):393-9.
    - Demonstrated the IHC compared to VI buffy coat
  - Grooms. Clin Diagn Lab Immunol. 2002 Jul;9(4):898-900.
    - Confirmed Njaa
  - Cornish. J Vet Diagn Invest. 2005 Mar;17(2):110-7.
    - Added ELISA and RT-PCR to validation

ELISA on PBS extracts

- Cornish. J Vet Diagn Invest. 2005 Mar;17(2):110-7.
  - Validated ELISA and IHC
    - Raised knowledge of acute positive skin
- Kuhne. J Vet Med B Infect Dis Vet Public Health. 2005 Aug;52(6):272-7
  - Validated ELISA on tag obtained samples compared to VI.
    - Demonstrated maternal antibody effect was minimal.

PCR approaches

- Ridpath. Virology 1994 Nov 15;205(1):66-74
- Kennedy. J Vet Diagn Invest. 2006 Jan;18:89-93
  - RT-PCR on Pooled Samples of Supernatant

Nebraska plan

- Evaluate the technique suggested by Kennedy in our lab on field samples
- Evaluate it using ELISA and IHC
- Estimate the load of virus in the extracts of positive samples
- Develop and evaluate logistical systems for implementation

Methods

- Routine customers with frequent positives were asked to ship samples fresh to the lab
- Samples were divided into groups of 100 based on order of arrival
- Extracts were made by immersion of biopsies in 2 ml of PBS/cm for a minimum of 10 minutes

- Pools of extract were made by pooling 100 ul of extract material
- RNA was extracted with Trizol
- Nested RT-PCR was performed
  - Sensitivity 2 TCID50/250ul
- ELISA's were performed with Idexx kit
- IHC was performed on formal in fixed notches fixed after PBS extraction
- 5,100 samples were tested

- Ten fold serial dilutions of PBS extracts from positive samples were submitted for PCR
- 51 pools were analyzed and true positive pools were defined as those positive on at least one sample by two assays (PCR, ELISA, IHC)
- Acute infections were not differentiated
- Negative pools with positive individual samples were evaluated for PCR inhibition by spiking pools and individual positive samples with cell culture virus

Summary Results

- 13 of 51 pools had at least one positive calf
- 10 positive by PCR on pool
- Inhibition of PCR in pool noted in 1 of 3 negatives
- One of three individual samples PCR neg
- Rate of inhibition in negative pools still being investigated

Distribution of Positive Calves Among Pools

- Positive pools
- Notches positive - # occurrences
  - One 8
  - Two 1
  - Three 2
  - Seven 1
- All but one individual sample positive by IHC was PCR positive.
- Negative pools each had a single positive calf

Pool Results			
# of Pools	pool PCR	individual sample IHC	Individual sample ELISA
7	Pos	Pos	Pos
2	Pos	Pos	Neg
1	Pos	Neg*	Neg
2	Neg	Pos	Pos
1	Neg	Pos	Neg
38	Neg	Neg	Neg

- Negative pool material was submitted to three other diagnostic facilities and was PCR negative at each of those facilities using their standard technique.
- Inhibition was investigated by spiking samples with 10 fold serial dilutions of cell culture virus from 2 to 200,000 TCID50
- Inhibition was also investigated by diluting individual sample with MEM

PBS dilution of positive samples

- Last positive dilution # of Occurrences
  - 1:10 0
  - 1:100 1
  - 1:1,000 12
  - 1:10,000 0
  - Amplification inconsistent at 1:2000 dilution These were the BVDV 1 isolates the 10 BVDV 2 isolate dilutions are in progress.

Conclusions

- Pooled sample PCR on ear notch extracts is not as sensitive as individual animal testing
- Pooled sample PCR is a sensitive and cost effective approach for use in feedlots
- Positive pools can be confirmed by IHC after PBS extraction or by ELISA on extracts.

- Simple dilution of samples is not responsible for sensitivity loss
- Work is needed to identify influences on sensitivity
- Sensitivity with each assay seems to be linked to rare individual animals or samples


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Spiked pool results		
Neg pool	**Positive sample**	**PCR result**
27 Sept. A	12501-06 #5187	Pos BVD1
27 Sept. B	12501-06 #26769	Neg run twice
27 Sept. C	12501-06 #26769	Pos BVD1
27 Sept. D	12501-06 #25151	Pos BVD1
27 Sept. E	12501-06 #25151	Neg run twice
27 Sept. F	12501-06 #11446	Neg run twice
23 Sept. B	12501-06 #11446	Pos BVD1
23 Sept. C	12501-06 #11446	Pos BVD1
28 Sept. A	12501-06 #11446	Pos BVD1

Individual test performance data

- 24 positive samples by two assays
  - 23 positive by IHC
    - Was faintly stained on original IHC slide
  - 23 positive by PCR
    - Repeated 3X and
  - 18 positive by ELISA
    - 6 negative