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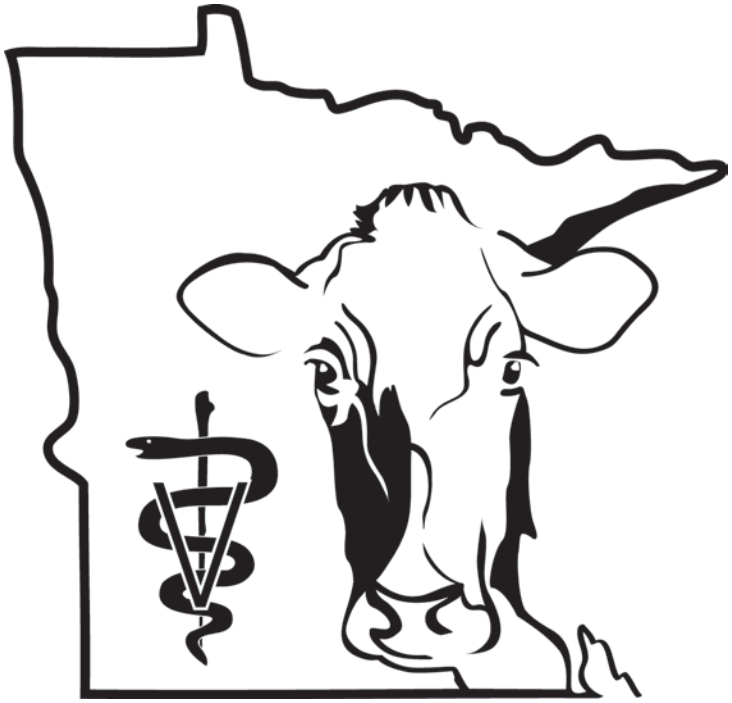

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DIAGNOSTIC METHODS USED TO DETECT BOVINE DISEASES

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Case Investigation

June 6, 2001

An aborted fetus and placenta from a closed, 45-cow dairy herd in east central Minnesota were submitted to the Veterinary Diagnostic Laboratory. The cows were kept in a tie stall barn and vaccinated with "9 way" and Brucella. The herd had been experiencing fertility problems. Laboratory findings from a fetal examination were negative.

August 16, 2001

An aborted fetus was delivered to the laboratory. Laboratory findings were negative.

August 31, 2001

An aborted, 6-months-gestational age bovine fetus, placenta and serum from the aborting dam (#53) were submitted. The fetus did not have gross or microscopic lesions. Bacteriologic examinations were negative including a negative examination for *Campylobacter sp.* Serum was negative for antibodies to *Neospora sp.*, leptospira, and bovine viral diarrhea (BVD) types 1 and 2. Serum from the dam and fetal tissues were positive for BVD by TaqMan^{R} polymerase chain reaction (PCR). A non-cytopathic strain of BVD was isolated from fetal tissue after one cell passage on bovine turbinate (BT) cells.

September 26, 2001

Convalescent serum and whole blood from cow #53 that aborted in August was submitted for serology and virus isolation. Virus isolation was negative. Serum was negative for antibodies to BVD type 1 and type 2 when tested by serum neutralization (SN).

October 31, 2001

Serum was collected from every animal in the herd (94 samples) and submitted for BVD TaqMan^{R} PCR. Cattle ranged in age from <1 year to 6 years. Seven of 94 samples were positive for BVD virus. Three heifers were less than 1-year-old, one cow was 2-years-old and three cows were 3-years-old.

November 15, 2001

Serum collected from the 7 BVD TaqMan^R PCR positive animals tested previously was again tested for BVD using TaqMan^R PCR. Each of the seven cattle tested positive for BVD confirming that they were persistently infected (PI). After eliminating BVD PI cattle from the herd, reproduction returned to normal. All newborn calves have been tested for BVD by TaqMan^R PCR to ensure that no PI calves reentered the herd. All calves to date have been negative for BVD virus.

Discussion

BVD, hog cholera of pigs and border disease virus of sheep are classified in the family Flaviviridae and are members of the genus *Pestivirus*. Disease caused by infection with BVD virus results in an estimated annual loss of $10 million to $40 million for every 1 million calves born.[1] Despite widespread use of BVD vaccination, the disease has proven difficult to control in some herds. Cattle persistently infected (PI) with BVD virus are the major reservoir for viral transmission within and between herds so eliminating PI animals and preventing their future entry into the herd is a critical part of herd health programs.

Several methods have been used to detect herds infected by BVD virus. In countries where BVD vaccination is uncommon, detection of BVD antibodies in bulk tank milk can be used to detect infected herds. Detection of BVD virus by PCR testing of bulk tank milk has been used, but the clinical usefulness of this approach has not been documented.[2] Also, results of bulk tank testing cannot be extrapolated to the young replacement heifers, calves and other animals that don't contribute milk to the bulk tank. The use of unvaccinated sentinel calves 6-12-months-old has been proposed as a cost effective way to identify BVD infected herds.[3] A herd was classified as likely to contain PI cattle when at least 3/5 heifers had SN antibody titers $\geq$ 128. The sensitivity of this method to classify BVD virus herd status was 66% and the specificity was 100%.

Historically, BVD virus detection involved cell culture isolation followed by virus detection through immunofluorescence or immunoperoxidase monolayer assay (IPMA) methods. More recently immunohistochemistry (IHC) has been suggested as a routine test for BVD virus. Results of recent experiments show that TaqMan^R BVD PCR is far more sensitive than IPMA, IHC and virus isolation.[4] The superior sensitivity and specificity of the TaqMan^R BVD PCR makes it a reliable and cost effective way of detecting and eliminating BVD PI cattle from dairy herds.

Comments:

1.) "Persistance" pays off. Don't get discouraged by negative laboratory results. Keep submitting fetuses and serum from aborting cattle for evaluation when the severity of the problem warrants continued investigation.
2.) Always attempt to include a full set of fetal specimens for evaluation. If maternal serum was submitted with the first abortion submission would BVD virus been detected earlier?
3.) BVD virus can persistently infect aged cattle.
4.) A history of being a "closed herd" is no assurance that the herd is free of BVD PI cattle.
5.) Cattle that are BVD positive by virus isolation or PCR should be retested a minimum of 2 weeks after the first test to ensure they are PI.
6.) IHC of ear notch skin does not distinguish PI from non-PI cattle.
7.) BVD PCR will detect modified-live-vaccine BVD virus. Wait a minimum of 3 weeks post vaccination before testing cattle for BVD virus by PCR.
8.) The sensitivity and specificity of the TaqMan^R BVD PCR makes it the single most reliable and cost effective way of detecting and eliminating BVD PI cattle from dairy herds.

References

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4. Mahlum CE, Haugerud S, Shivers JL, et al. Detection of bovine viral diarrhea virus by TaqMan^R reverse transcription polymerase chain reaction. *J Vet Diagn Invest* 2002;14:120-125.