PEER REVIEWED

A Preliminary Evaluation of the Effect of Vaccination with Modified Live Bovine Viral Diarrhea Virus (BVDV) on Detection of BVDV Antigen in Skin Biopsies Using Immunohistochemical Methods

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Abstract

Immunohistochemical (IHC) testing of skin biopsies (ear notch samples) is a method to identify cattle persistently infected (PI) with bovine viral diarrhea virus (BVDV). Skin biopsies were taken from 90 calves upon arrival (day 0) at a feedlot, and on days 14, 28 and 42 following arrival. Calves were vaccinated with modified live virus (MLV) BVDV vaccine on days 1 and 14 following arrival. Additional samples were taken from 50 of the calves between days 1 and 10 when they were treated for bovine respiratory disease. Immunohistochemical testing for BVDV antigen was performed on all samples.

One calf was positive for BVDV antigen by IHC techniques on 5 skin biopsies. This calf was also positive by virus isolation (VI) on the buffy coat of a blood sample taken on day 42 following arrival. All samples from the remaining calves were negative when tested with the IHC method.

Other tests, VI and polymerase chain reaction (PCR), used to identify PI cattle may give positive results in cattle recently vaccinated with MLV BVDV vaccine. This study suggests that IHC testing of skin biopsies may be advantageous over other tests to identify PI cattle recently vaccinated with MLV BVDV. Further studies are needed to better define the utility of this testing method for cattle that are PI with BVDV.

Introduction

Bovine viral diarrhea virus (BVDV) infection is one of the most economically important diseases affecting the cattle industry. Identifying persistently infected (PI) animals which remain viremic and shed virus throughout their lifetime is an important step in any BVDV control program.

Erosive skin lesions are a common manifestation of mucosal disease in PI cattle.[1,14,16] The presence of BVDV antigen in keratinocytes in the stratum basale and stratum spinosum of the skin has been documented in clinically normal PI cattle.[2,3,4] This has lead to immunohistochemical (IHC) testing of skin biopsies (in the form of ear notch specimens) as a diagnostic tool for detecting PI animals. Previous studies have shown IHC testing of skin biopsies to be as reliable for detecting PI cattle as virus isolation (VI) (standard or immunoperoxidase microtiter plate) performed on serum or whole blood samples.[6,7,15]

Immunohistochemical testing has several advantages over other screening methods used to identify PI cattle. Virus isolation using serum or whole blood can be affected by colostral immunoglobulins. Testing should only be done on calves over 2 months of age.[5,11] Immunohistochemical antigen detection for BVDV is not affected by colostral immunoglobulins.[11] Once fixed in formalin, skin samples do not require special handling, such as refrigeration for storage or shipment. Collection of skin biopsies requires no special skills, such as venipuncture, and therefore, can be done by the producer or an employee. Immunohistochemical testing is economically feasible and typically more rapid than VI.

Transient viremia can be seen for up to 2 weeks following vaccination with modified live virus (MLV) BVDV vaccine.[8,9,12,13] The vaccine virus has been isolated from blood samples obtained from recently vaccinated cattle.[8,9] Polymerase chain reaction testing can detect nucleic acid from the vaccine virus, resulting in false positives.

BVDV viral antigen has been detected by IHC methods in ovarian tissue up to thirty days after administration of MLV vaccine.[9] Consequently, the possibility of false positives occurring when using IHC testing on skin biopsies following vaccination with MLV vaccine does exist. This has not been studied. The purpose of this study was to determine whether vaccination with MLV BVDV vaccine affects the outcome of IHC testing on skin biopsies for BVDV.

Materials and Methods

Ninety crossbred beef heifers weighing an average of 457 lb (208 kg) were purchased from livestock auctions in southern Oklahoma and northern Texas. At arrival, each calf was individually identified with a numbered ear tag and a skin biopsy (ear notch sample) was taken and placed in 10% buffered neutral formalin (BNF). All biopsies were taken from the ventral margin of the pinna. Triangular shaped skin samples were taken with a pig ear notcher and measured 0.25 in (base) by 0.5 in (sides).

Eighteen hours after arrival each animal was vaccinated with an intramuscular multivalent MLV vaccine[a] which included BVDV. Calves were evaluated daily for clinical signs of bovine respiratory disease, including labored breathing, lethargy, weakness, depression, and anorexia. Calves displaying clinical signs were removed from their pens and taken to the treatment facility. Those with a rectal temperature greater than 104°F (40°C) were treated with an antimicrobial according to the protocol used at the feedlot. Skin biopsies were collected from each calf when it was initially treated, and additional samples were collected if the calf was retreated. Fourteen days following arrival the calves were re-vaccinated with the same multivalent MLV vaccine. Skin samples were collected from all 90 calves at 14, 28, and 42 days following arrival and placed in 10% BNF.

Skin biopsies were routinely processed, embedded in paraffin blocks, and sectioned. Five micron sections were placed on positively charged glass slides and stained by immunohistochemical procedures routinely performed for detection of BVDV antigen at the Oklahoma Animal Disease Diagnostic Laboratory.

Whole blood was collected in an EDTA tube at 42 days following arrival from the calf which was positive when previously tested using IHC. Routine BVDV isolation from the buffy coat layer was performed.

Results

All 90 calves were sampled at least 4 times for IHC testing. Sixty-one additional skin samples were taken from 50 of the calves within the first 10 days following arrival. One calf was positive for BVDV by IHC methods; five samples from this calf were positive. Virus isolation from the buffy coat of the blood sample from this calf on day 42 was also positive for BVDV. Samples from the remaining 89 calves were negative by IHC testing. Results of all IHC testing is summarized in Table 1.

Discussion

Results of this study suggest that MLV BVDV vaccination does not result in adequate deposition of viral antigen in the skin to be detected by IHC methods. These findings are similar to those in another study where skin biopsies from 22 calves experimentally infected with BVDV were all negative when tested by IHC

Table 1. Results of IHC testing of skin samples at different days following arrival

Day	0	3	4	5	6	7	8	9	10	14	28	42
No. calves	90	20	6	16	3	2	9	3	2	90	90	90
Neg	89	19	6	16	3	2	9	3	2	89	89	89
Pos	1*	1*	0	0	0	0	0	0	0	1*	1*	1*

*Sample from calf number 354.

methods.[6] In our study, vaccination with MLV vaccine did not appear to interfere with our ability to identify the PI calf in this group with IHC tests.

The possibility exists that the MLV BVDV used in these cattle was not viable. In retrospect, vaccine viability should have been confirmed. The vaccine was stored, handled, and administered in accordance with the manufacturer's directions by skilled, trained personnel. It is also possible that the cattle in this study did not respond to vaccination due to pre-existing immunity to BVDV. It is unlikely that a high percentage of lightweight calves purchased through a livestock market were immune to BVDV. In fact, one investigator reported that 74% of calves similar to those in our study arriving at a research feedlot did not have an antibody titer against BVDV.[10] Future studies should consider doing parallel BVDV serology, virus isolation, and IHC testing to rule out these possibilities.

The findings in our preliminary study may not be applicable to other commercially available BVDV vaccines, since vaccine strain and antigenic mass vary between companies. In future studies, several vaccine lines should be included.

Conclusions

The findings in this study suggest that vaccination with MLV BVDV vaccine does not cause false positives when IHC testing is used on skin biopsies. This implies that IHC testing of skin samples may be preferred over other tests to screen for PI individuals in recently vaccinated cattle or in cattle purchased with unknown vaccination histories. Further studies are suggested to better define the advantages and shortfalls of IHC testing of skin samples to screen for PI cattle.

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Footnote

[a]BRSV-Vac 4, Bayer Corp., Shawnee Mission, KS.

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