

Laboratory Diagnosis of Virus Infections in the Feedlot

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Introduction

Laboratory confirmation of clinical diagnosis can serve an important role in the maintenance of a health program in the feedlot. There is an abundance of scientific literature and review articles documenting the involvement of viruses in bovine respiratory disease.^{1,2} Laboratory tests can supply information on which viruses are involved in causing clinical disease. This information can assist in making informed decisions for future vaccination, treatment and herd health programs. Routine surveillance testing can supply data on the changing disease conditions of a feedlot and assist in maintaining client satisfaction.

To obtain good diagnostic laboratory results it is important that the appropriate samples are collected early in the course of the disease. The samples should be chosen according to the clinical conditions and the differential diagnosis. Due to the time required in conducting laboratory tests and getting the complete results, all possibilities should be thoroughly anticipated in submitting samples to assure that the laboratory results will assist in the diagnosis.

This report summarizes some of the available laboratory tests and their utilization for diagnosis of viral infections in the feedlot.

Laboratory Test

Virus Isolation:

Virus isolation in cell culture is routinely used in many laboratories to detect viruses that are of importance in the feedlot. Most viruses recognized as contributing to respiratory disease in the feedlot can be isolated on cell culture. Certain viruses are readily isolated on cell culture while others require special conditions for cultivation and identification. When a virus is indicated in the inoculated cells, additional tests are required to identify the specific virus. The type of testing performed after a virus is detected depends on the specific virus and the type of cytopathic effect (CPE) that is observed. For example, infectious bovine rhinotracheitis (IBR) normally produces a characteristic CPE and can be confirmed by using fluorescent antibody specific for IBR. In contrast, non-cytopathic bovine virus diarrhea (BVD) does not produce a noticeable change in cell

culture and is detected by performing a fluorescent antibody test using BVD specific fluorescent antibody on inoculated cell culture after 5-7 days of incubation. The routine use of cell culture in diagnostic virology also serves as a surveillance system to identify new or previously unrecognized viruses.

Fluorescent Antibody Test

The fluorescent antibody (FA) test is used to detect specific antigens. Fluorescently conjugated antibody specific for a particular antigen is allowed to react with the specimen and the antibody specific reaction is detected using a fluorescent microscope. Specific fluorescent conjugates are available for most bovine viruses recognized to be important to bovine respiratory disease. Direct fluorescent antibody detection of many viruses can be achieved by reacting individual specific conjugates with frozen sections of tissue and observing for characteristic fluorescence.

Enzyme-Linked Immunosorbent Assay (ELISA)

The use of ELISA technology has expanded rapidly in recent years and is available for direct detection of virus antigens. Commercial test kits utilizing monoclonal antibody have proven to be a specific and sensitive means of detecting virus antigens. A commercial ELISA test for Human Respiratory Syncytial Viruses (RSV) has been shown to react with Bovine RSV³ and is routinely used in our laboratory to identify RSV.

Hemagglutination

Certain viruses have the ability to react with erythrocytes and cause the erythrocytes to agglutinate. Erythrocytes will also adsorb to the surface of certain virus infected cell cultures and can be visualized by light microscopy. This adsorption of erythrocytes to virus infected cells is termed hemadsorption and can assist in identification of bovine parainfluenza-3 (PI#) and bovine parvovirus.

Electron Microscopy

Electron microscopy is routinely utilized for those viruses that are difficult to recover by conventional virus de-

tection procedures. Direct observation of viruses by electron microscopy can allow rapid identification of viruses. The use is, however, restricted to those viruses that produce sufficient quantities of viruses for detection. Electron microscopy can assist in the identification of new or previously unidentified viruses and also for those viruses for which no fluorescent conjugate or antiserum is available. Picornavirus isolates from cell culture are identified by electron microscopy.

Serology

The serum virus neutralization (SN) test can adequately indicate exposure history or vaccination status of animals to the bovine respiratory disease viruses. After virulence virus exposure most animals produce significant antibody titers that will persist for the feeding period. Additional test procedures that are used to detect the presence of virus specific antibody include hemagglutination inhibition (HI), complement fixation (CF) and enzyme-linked immunoadsorbent assay (ELISA). The construction and use of ELISA test that can identify antibody activity in specific immunoglobulin classes and antibody directed against specific virus antigens can assist in determining time of exposure and/or vaccination status.

Results

Table 1 shows the virus neutralization titers obtained on sera submitted over a two year period to the Texas Veterinary Diagnostic Laboratory at Amarillo. Of the sera tested for IBR antibody 54.4% were negative with a titer of <4. Forty-one percent of the sera had <4 titers for BVD. The percent negative for RSV and PI3 were 34.3% and 26.7% respectively. These percentages are similar to previous years with the exception that PI3 has shown a slight increase in the number of negative titers detected.⁴

Table 1. Summary of Serum Virus Neutralization Titers for 1989 and 1990

Number tested	VIRUS			
	IBR	BVD	RSV	PI3
TITER	%	%	%	%
<4	54.4	41.3	34.3	26.7
4	10.5	6.3	12.4	8.4
8	9.9	6.6	13.9	9.0
16	9.3	8.1	12.6	10.0
32	6.8	9.9	9.9	12.1
64	3.9	9.1	7.2	10.7
128	1.9	8.6	4.6	11.5
>256	0.8	7.2	2.3	10.5
TOXIC	2.6	2.9	2.8	1.2

Table 2 summarizes virus isolation results obtained on specimens submitted to the Diagnostic Laboratory at Amarillo over a nine year period. These results do not include samples that were submitted or tested for RSV identification. Diagnostic requests for RSV identification are performed by a commercial ELISA procedure and these results are not included in virus isolation. Viruses were re-

Table 2. Summary of Virus Isolation

	Number	VIRUS					TOTAL POSITIVE
		IBR	BVD	PI3	PICORNA	OTHER	
ACCESS %	8549	730 8.5	859 10.1	112 1.3	61 0.7	74 0.8	1836 21.5
ANIMAL %	15727	1022 6.5	1085 6.9	159 1.0	75 0.5	85 0.5	2426 15.4
TISSUE %	34614	1561 4.5	1641 4.7	195 0.6	85 0.3	119 0.3	3601 10.4

covered from 21.5% of the 8,549 accessions submitted for virus isolation. Many of the accessions had multiple animals with multiple tissues tested for each animal. The percent of animals positive for virus was 15.4% with 10.4% of the tissues testing positive. The major viruses recovered from the submitted accessions were IBR at 8.5% and BVD at 10.1%. The ability to recover viruses on submitted specimens is highly dependent upon the time of sample collection, the quality of sample collected and the arrival time at the laboratory.

In contrast to the overall virus isolation results obtained over a nine year period, Table 3 shows the isolation results from 101 calves showing acute clinical signs of respiratory illness. Nasal swabs and heparinized blood were collected on the first day of clinical illness and immediately refrigerated (blood) or frozen (swabs). Samples were collected over a 14 day period from one feedyard and received at the laboratory within 24 hours of collection. Nasal swabs and buffy coat preparations were inoculated on cell culture and observed for 5 to 7 days. Nasal swabs were also tested for RSV by a commercial ELISA test. The overall recovery rate of virus from this group of calves was 61.4%. Multiple viruses were detected in 18 of the 62 calves found positive.

Table 3. Viruses Recovered Compared to Days on Feed

Days On Feed	Total Calves	Buffy Coat		Nasal Swab				Total Positive Calves	
		IBR	BVD	IBR	BVD	PI3	RSV		Picornia
1-14 %	67	1 1.5	18 26.9	12 17.9	29 43.3	19 28.4	7 10.4	0 0.0	48 71.6
15-120 %	34	0 0.0	0 0.0	10 20.8	2 5.9	2 1.9	1 2.9	1 2.9	14 41.2
Total %	101	1 1.0	18 17.8	22 21.8	31 30.7	20 19.8	8 7.9	1 1.0	62 61.4

Conclusion

Virus recovery can implicate a virus as contributing to clinical respiratory disease in the feedlot. When specimens are collected, transported and tested under optimal conditions both positive and negative virus isolation results can be considered significant.

References

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Abstracts

An economic assessment of twin births in British dairy herds

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Veterinary Record (1991) 129, 526-529

The effect of twinning on the subsequent health, production and reproductive performance of dairy cattle was studied by analysing the data derived from 19,755 calvings which occurred during three years on 37 farms. The data formed part of the database of a veterinary practice operating the DAISY dairy cow recording scheme for its dairy farmer clients. The average twinning rate was 2.5 per cent. For first calf heifers the rate was 0.9 per cent, and the rate increased with increasing parity to over 5 per cent for cows calving for their sixth and subsequent lactations. Although they produced more milk than their contemporaries, twin-bearing cows suffered an increased incidence of retained placenta and vulval discharges and their calving to conception interval was extended by 33 days. Furthermore, 35 per cent of these cows were culled compared with 21 per cent of their contemporaries. The benefit of having more calves for sale was reduced owing to 15 per cent of them being born dead. It is calculated that producing twins resulted in an average loss of income of £ 74/cow, a deficit of 15 per cent compared with cows having single calves.

Efficacy of tilmicosin in treatment of pulmonary infections in calves

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The efficacy of tilmicosin in the treatment of respiratory infections in calves was evaluated. According to a randomised block design, 58 calves with naturally occurring respiratory infections were treated with one of the following products: a single subcutaneous injection of tilmicosin (10 mg/kg liveweight) or daily intramuscular injections of 5 mg lincomycin and 10 mg spectinomycin/kg liveweight, for a minimum of three days. Both treatment groups initially showed similar clinical signs and their initial responses to the treatments were good. However, the tilmicosin treated calves improved more rapidly. Significantly greater improvements ($P \leq 0.05$) were observed in their demeanour and appetite during the first 10 days after treatment began, and in their respiratory condition between five and 10 days after treatment began.