treatment rates were numerically ($P \geq 0.05$) lower in pens categorized as positive for Type II BVDV infection at feedlot arrival as compared to negative pens.

At the individual animal level, acute BVDV infections, defined as BVDV viremia either at the time of arrival or at the time of initial diagnosis of UF or NF, were associated with a significantly ($P < 0.05$) increased risk of overall mortality and infectious disease mortality. However, these associations were more consistently observed with Type I BVDV than with Type II BVDV. Note that Type I BVDV viremia was only detected at the time of initial diagnosis in 4.00% of UF cases and 2.55% of NF cases. Similarly, Type II BVDV viraemia was only detected at the time of initial diagnosis in 2.50% of UF cases and 1.70% of NF cases. In the mortality-based BVDV testing survey, evidence of BVDV infection was only found in 5.56% of non-PI animals.

## Significance

In summary, the results of this study are in general agreement with previous seroepidemiologic work that has demonstrated highly variable correlations between pen-level evidence of BVDV infection and animal health outcome. However, the differences observed between Type I BVDV viremia and Type II BVDV viremia on pen-level morbidity and mortality and the effect of acute BVDV infections on the risk of individual animal mortality have not been previously described. Based on the overall findings of this study, BVDV infection in non-PI animals has occurred less frequently than BVDV infection in previously studies. This observation may have been a result of the BVDV vaccination program used in the study.

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# Bovine Respiratory Syncytial Virus, Bovine Coronavirus, and Bovine Viral Diarrhea Virus Diagnosis by PCR Testing of Nasal Swabs: Comparison with Cell Culture Procedures

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## Introduction

Bovine respiratory diseases (BRD) also includes "shipping fever" among its several disease manifestations. "Shipping Fever" may be caused by *Mannheimia haemolytica*, *Pasteurella multocida* and occasionally *Histophilus somni*, as well as viral agents including infectious bovine rhinotracheitis virus (bovine herpesvirus-1 [BHV-1], bovine viral diarrhea viruses (BVDV1a, BVDV1b, BVDV2a), bovine parainfluenza-3 virus (PI-3V) and bovine respiratory syncytial virus (BRSV). These viral antigens are in the viral vaccines used to control BRD. These viruses may cause respiratory tract infection and disease but often predispose the cattle to the bacterial pathogens. Additional viruses associated with BRD include bovine coronavirus (BCV) and bovine adenoviruses (BAV) with multiple serotypes.

Diagnosis of viral infections has traditionally used viral isolation in cell culture and serologic testing (antibody levels) to detect active infection (rising antibody levels). These procedures require considerable time for the results, especially cell culture isolation (days to weeks for multiple passages). Serology requires the host response post infection (3-4 weeks for acute to convalescent samples), plus the antibody test performance. Use of cell culture systems for viral diagnostics is useful for selected viruses, yet some viruses may not be readily identified in cell culture or may require unique cell cul-

tures not normally maintained in diagnostic laboratories. Also, some viruses may be quite labile and have reduced chance of recovery due to time and conditions of shipment. There are a limited number of rapid antigen detection systems available for diagnoses of viruses causing BRD. An antigen capture ELISA test for BVDV, used primarily as a test for persistently infected (PI) BVDV cattle using fresh ear notches, is a newer and more rapid test.

Molecular diagnostic testing, such as reverse transcriptase polymerase chain reaction (RT-PCR), offers a rapid means and greater sensitivity than the traditional tests like cell culture assay and serology. The nucleotide sequences for primer selection for the RT-PCR are available. The purpose of this study was two-fold: 1) implement RT-PCR tests for BRSV, BCV and BVDV using nasal swab samples from cattle; and 2) compare the RT-PCR results for BRSV, BCV and BVDV with cell culture assay results.

## Materials and Methods

Nasal swabs were collected from cattle using a commercial culturette. The nasal swabs were then placed in 2 ml of cell culture media and frozen until assayed by cell culture testing and RT-PCR.

Cattle utilized in the study represented three different groups: 1) cattle were from a study of susceptible calves exposed to BVDV PI calves with serum, PBL and nasal swabs collected at day 0, and then weekly thereafter till day 35. Calves were identified as acutely infected based on serology (4-fold increase in antibody levels) and/or viral isolation; 2) BVDV PI calves were available with samples collected including nasal swabs; and 3) cattle in a feedlot were studied for BRD agent identification while in the initial month post-arrival, including 20 sentinel calves with acute and convalescent samples and seven calves in the sick pen with acute and convalescent collections.

Cell culture assays were of two methods: 1) qualitative cell culture assay (QCCA) based on identification (positive or negative) in bovine monolayer cultures with two passages and observation of viral CPE, and staining for NCP BVDV; and 2) quantitative assay using viral titration (VT) in 96-well plates for NCP BVDV using MDBK cells.

A nested multiplex RT-PCR assay was used to detect BVDV, an RT-PCR assay was used to detect BCV, and a nested RT-PCR assay was used for BRSV.

## Results

In the first study, the RT-PCR detected BVDV in nasal swabs of selected acutely infected calves. There were nine acutely infected calves that were negative for BVDV RT- PCR, and all nine were negative by the QCCA in the nasal swabs. There were, however, five acutely infected calves negative for BVDV by the QCCA, yet the five calves were positive by BVDV RT-PCR in the nasal swabs. Thus in the nasal swabs, BVDV RT-PCR detected virus when the QCCA did not.

The BVDV RT-PCR in nasal swabs of BVDV PI calves was positive in all 32 calves. The viral infectivity in cell cultures with nasal swab inoculation was either by the QCCA or the VT. There were eight PI calves positive both by the RT-PCR and QCCA in nasal swabs. There were the remaining 24 PI calves with VT results available for 11 calves. There were two calves' nasal swab samples which were toxic to the cell cultures at undiluted, yet they were RT-PCR BVDV positive. There were 11 PI calves with VT titers ranging from $10^{3.65}$ to $10^{4.9}$ TCID50 per ml of nasal swab. These results suggest RT-PCR for BVDV detects viruses in some samples toxic to cell cultures.

There were nasal swab samples from 27 calves in a feedlot during an investigation for BRD etiologic agents with both acute and convalescent samples available from 20 sentinel calves (collected at entry and 30 days later), and there were seven calves in the sick pen with acute samples and convalescent samples collected two weeks later. All nasal swabs from the 27 calves for both acute and convalescent collections were negative for viruses causing CPE and negative for NCP BVDV after two passages in the QCCA. There was only 1/20 sentinel calves positive for BRSV RT- PCR at the acute collection, with 4/12 positive by BRSV RT-PCR at the convalescent collection (eight calves were not tested). There were 5/7 sick pen calves positive for BRSV RT-PCR at the acute collection, and 4/6 positive at the convalescent sample (one calf died) two weeks later. The four positive sick pen positive calves at convalescent sampling were also positive by BRSV RT-PCR at the initial collection. There were 2/20 sentinel calves positive for BCV RT-PCR at the initial collection and 0/20 positive at the convalescent collection. All seven sick pen calves were BCV RT-PCR negative at the initial collection, and none of six remaining calves were BCV RT-PCR positive at the convalescent collection. In this BRD study, it appears that BRSV infections increased from acute to convalescent sampling, and BRSV was present in the majority of sick pen calves over the two week collection interval. Also, the RT-PCR assays were more efficient than QCCA in detecting positive samples for both BRSV and BCV.

## Significance

These results indicate that molecular diagnostic procedures, such as RT-PCR, provide identification of viruses such as BRSV, BCV and BVDV when cell cul-

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ture assays are negative for these viruses. For BVDV, the BVDV RT-PCR identifies virus at levels in the nasal swabs below the detection in the QCCA. Also, the RT-PCR can be performed in hours compared to days in the cell culture assays.

Potentially, use of the RT-PCR and other molecular tests could provide a more rapid detection of viral pathogens than traditional cell culture assays, as well as identifying agents not regularly found by cell culture assays. Information gained by these molecular diagnostic procedures may assist the clinician in making decisions for BRD control and management. This is illustrated by the detection of BRSV in nasal swab collection, whereas cell culture assay was negative for BRSV.

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# Analysis of the Association between ELISA and Nested PCR on Blood and Milk for *Mycobacterium avium subsp. paratuberculosis* Detection in Holstein Cows

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## Introduction

Paratuberculosis is a chronic, infectious disease of ruminants characterized by progressive weight loss and profuse diarrhea. It is caused by the acid-fast bacillus *Mycobacterium avium* subspecies *paratuberculosis* (MAP), has a worldwide distribution and is categorized by the OIE as a List B disease that has serious economic impact or is a public health concern. Disease diagnosis is hampered by a lack of sensitive tests. Available methods fail to identify all infected animals, and many produce substantial numbers of false positives and false negatives. This is of particular importance, as they relate to detection of the organism in subclinically infected animals. Several commercial, enzyme-linked immunosorbent assay (ELISA) tests are available, but it is generally accepted that their sensitivity in detecting infected animals is only about 50%. In fact, because of its low sensitivity, the ELISA test is rarely positive in animals under two years of age. Polymerase chain reaction (PCR) tests based on the insertion element IS900 have been the most widely used for MAP identification. However, detection of the etiologic agent is limited by frequency and number of organisms present in the fluid or tissue. A combination of serologic tests, such as ELISA, and agent detection through nested PCR could be a useful strategy to improve the sensitivity of paratuberculosis diagnosis, especially when detectable target levels for each test follow different temporal patterns.

The objective of this study was to compare the performance of ELISA testing of sera, and nested PCR in milk and blood, for diagnosis of paratuberculosis in Holstein cows.

## Materials and Methods

Blood and milk samples were collected from 256 Holstein cows in three dairies near Gainesville, Florida. The ELISA originally developed by WD Richards (Allied Laboratories, Inc, Ames, IA) was performed with crude, soluble protoplasmic antigen (Allied Monitor Missouri). Test sera were pre-absorbed over night with *Mycobacterium phlei*. ELISA results were calculated from wavelength readings (OD at 405 nm) of triplicates and recorded as negative (<1.5 OD), suspicious (1.5 to 1.9 OD) and positive (>2.0 OD). For PCR analysis, milk and blood samples were probed with primers P90, P91 for IS900, which specifically recognizes a 413 bp se-