Potentiation of Bovine Respiratory Syncytial Virus Infection in Calves by Bovine Viral Diarrhea Virus

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Abstract

The potential synergistic effects of BVDV and BRSV infections were investigated. Single virus infections often produce mild, if any, clinical disease in controlled animal challenge studies. The multi-etiologic nature of bovine respiratory disease is well-documented, and the potential effects of dual infection more closely reproduces natural disease and the significance of pathogenic viruses. This is important in designing vaccination programs for disease control.

Fourteen Hereford beef calves weighing approximately 205 kilograms were obtained from a ranch in western Nebraska. The calves were seronegative to IBR, BVD, PI3, and BRSV, and were confirmed to be free of persistent BVD infection through repeated negative virus isolation. Random assignment to form treatment groups allotted four calves to a BVD challenge alone, four calves to a BRSV challenge alone, four calves to dual BVD, BRSV challenge, and two calves to a negative control group. Evaluation methods included pre-mortem clinical scoring, hematology, and virus isolation. Euthanatized calves were evaluated by virus isolation, gross pathologic, and histopathologic evaluation.

Severity of clinical signs (depression, serous to mucopurulent nasal discharge, and dyspnea) and extent of lung injury was greater in calves inoculated with dual BRSV/BVDV than in calves inoculated with either virus alone. Potentiation of BVDV infection of the digestive tract by BRSV was indicated by augmentation of clinical diarrhea in calves dually-infected with BRSV and BVDV compared to calves infected only with BVDV. Gross and histopathologic lesions correlated to pre-mortem clinical observations.

BVDV and BRSV potentiate each other in dual virus infection, causing more severe clinical signs and lesions than in infection with either virus alone. This more closely mimics natural infections, and better establishes the clinical significance of these pathogens than single virus controlled challenges studies.

Introduction

Bovine respiratory disease (BRD), a major health problem of cattle in the United States, causes greater economic loss than all other feedlot diseases of cattle combined. Complex interactions between viruses, bacteria, mycoplasmas and various environmental stressors of calves cause clinically severe BRD. Severe pneumonia in feeder cattle is frequently associated with mixed bacterial and viral infections. Pathogenic bacteria are frequently carried in the nasopharynx of normal cattle where they reside as commensals. The respiratory system of normal cattle is highly resistant to bacterial colonization. When the mucociliary apparatus becomes impaired by viruses such as bovine respiratory syncytial virus (BRSV), respiratory secretions increase in amount and viscosity which reduces the efficiency of this defense mechanism. Degeneration and necrosis of ciliated epithelial cells caused by BRSV or other viral infection follows and further inhibits the ability of the tracheobronchial tree to remove foreign material including bacterial pathogens. Subsequently, efficiency of pulmonary macrophages to clear the lung of bacterial pathogens is suppressed resulting in colonization and proliferation of bacterial pathogens such as *Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus,* or *Actinomyces pyogenes*. Mixed bacterial and BRSV infections have been shown experimentally to cause more severe respiratory disease.[1] It has also been demonstrated that mixed viral infections play a role in BRD both in natural and experimental infections. There is serologic evidence of naturally-occurring mixed viral infections in BRD where seroconversion to BRSV was associated with an increased rate of seroconversion to infectious bovine rhinotracheitis virus (IBR), parainfluenza type 3 virus, bovine adenovirus type 3, and BVDV.[2] Bovine virus diarrhea virus infection has been shown to result in increased distribution of IBR virus in experimentally inoculated calves.[3] The etiologic role of BVDV in BRD has been attributed to its immunosup-

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pressive effects in the host animal since there is limited[4] or no[5] evidence for lung injury due to BVDV infection. Bovine viral diarrhea virus has a tropism for lymphoid cells of the primary and secondary organs of the immune system.[6] The virus causes decreases in populations of those cells *in vivo*[7,8,9] as well as down-regulating cell function *in vitro*.[10,11,12,13] Immunosuppression has been associated with increased susceptibility of cattle to bacterial infections.[14] Bovine respiratory syncytial virus causes bronchitis, bronchiolitis and pneumonia.[15,16,17] While individual effects of BVDV and BRSV on the host animal are well documented, synergistic effects of simultaneous BVDV and BRSV infections have not been defined. The objective of the present study was to determine the pathologic effects of concurrent BRSV and BVDV infections in calves to determine if disease-potentiation occurred during simultaneous infections.

Materials and Methods

Calves

Fourteen yearling Hereford beef calves, serologically and virus isolation negative for BVDV and BRSV, weighing approximately 205 kg were obtained from a privately-owned range herd. The calves were transported to the Animal Research Facility at UNL and were housed in BL2 isolation rooms (2 calves per room). Animals were fed a commercial complete pelleted ration with continuous free access to fresh water. The calves were acclimated for 7 days before initiating the study.

Experimental design

The calves were randomly assigned to 1 of 4 treatment groups: group 1. Calf numbers 1, 2, 3, 4; group 2. Calf numbers 5, 6, 7, 8; group 3. Calf numbers 9, 10, 11, 12; group 4. Calf numbers 13, 14. Calves in group 1 were exposed to BVDV and calves in group 2 to BRSV. Calves in group 3 were exposed to both BRSV and BVDV and calves in group 4 were non-inoculated controls. Calves were euthanatized and necropsied as follows: Day 3: Calf numbers 1, 5 and 9; Day 6: Calf numbers 2, 6, 10 and 13; Day 9 : Calf numbers 3, 7 and 11; and, Day 12: Calf numbers 4, 8, 12 and 14.

Viruses

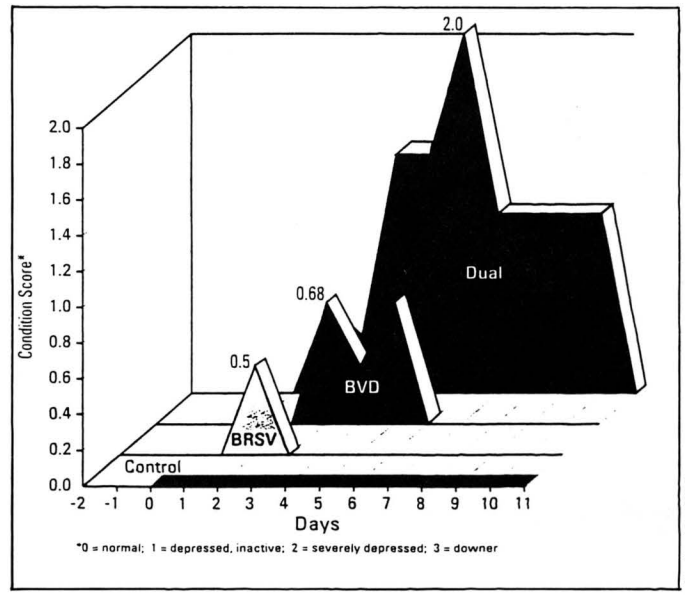
Calves were inoculated intranasally with 2 x 10^8 tissue culture infective doses 50% ($TCID_{50}$) of New York-1 BVDV on day 0. Bovine respiratory syncytial virus challenge virus (Isolate No. 165, 1 x $10^{5,9}$ $TCID_{50}$ /ml provided by M. O'Hara and L. Nelson, Pfizer Animal Health) was administered intranasally (5 ml) and intratracheally (5 ml) in the morning, and intranasally (5 ml) in the afternoon on Days 0, 1 and 2.

Clinical Evaluation

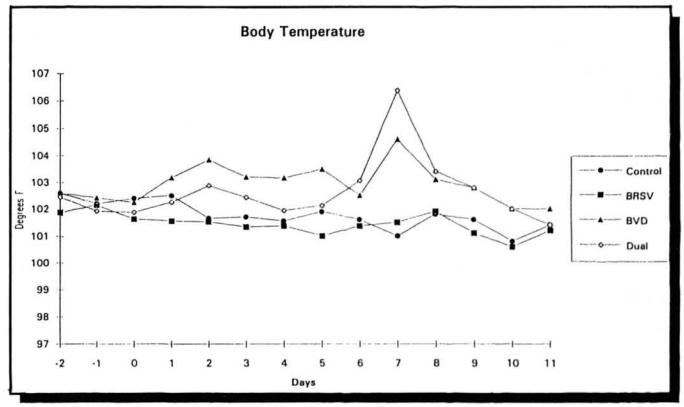
Each calf was evaluated clinically and the body temperature of each calf was determined twice daily. The following clinical parameters were evaluated and scored: (1) The amount and character of the nasal discharge (0-normal; 1-excessive serous; 2-copious mucopurulent); (2) Respiratory rate and character (0-normal; 1-rapid, shallow; 2-rapid, shallow, occasional cough; 3-labored, dyspneic, cough); (3) Consistency of feces (0-normal; 1-soft, non-formed; 2-watery, semi-solid; 3-liquid); (4) Appetite (1-anorexic; 2-dehydrated); and (5) Condition (0-normal; 1-depressed, inactive; 2-severely depressed; 3-nonambulatory).

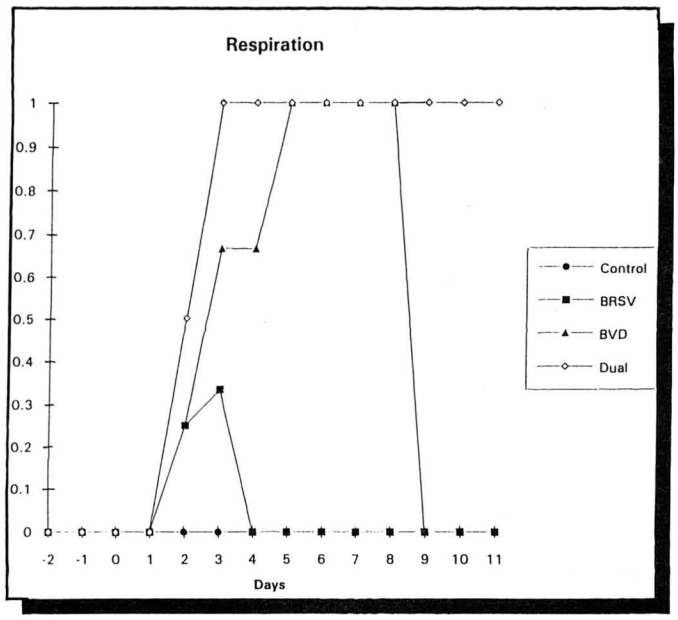
Post-mortem examination

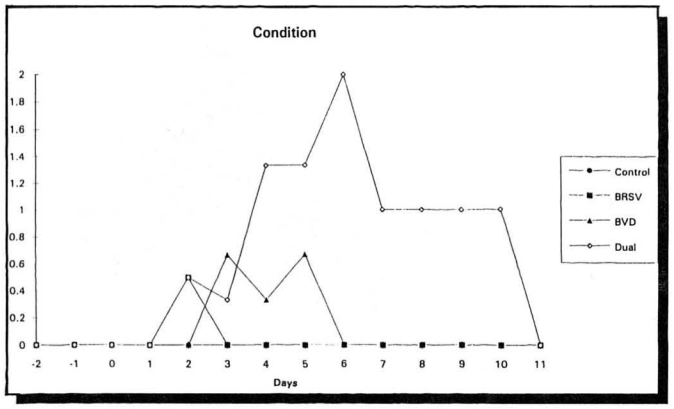
Calves were euthanatized, necropsied and tissues were collected for virus isolation, bacteriologic, histologic and immunohistochemical examination.



Mean condition of challenge and control calves throughout study.







Results

Clinical findings

Three (numbers 2, 3, and 4) of the four group 1 calves (numbers 1, 2, 3 and 4), exposed with BVDV only, exhibited moderate clinical signs. Calf number 1, which was euthanatized and necropsied on day 3, did not exhibit clinical signs that distinguished it from the control calves (numbers 13 and 14). Three calves (numbers 2, 3 and 4), exhibited clinical disease signs, beginning on Day 3 and continuing until the day of necropsy, consisting of the following: excessive serous nasal discharge; rapid, shallow breathing patterns; soft, abnormal fecal consistency ranging from non-forming to liquid feces; anorexia; and, generalized depression and inactivity.

Three (numbers 5, 6 and 7) of 4 calves inoculated with BRSV (group 2) developed mild clinical signs on Day 2 or 3 consisting of excessive serous nasal discharge. Calf numbers 5 and 6 had rapid, shallow respiration on Day 2. Two calves (numbers 5 and 6) were depressed and inactive on Day 2. Calf number 8 had a rapid respiratory rate on Day 11.

All 4 calves in group 3, dual-infected with BRSV and BVDV developed pronounced clinical disease beginning on Day 2 and continuing to the day of necropsy. Clinical signs included excessive nasal discharge of serous (Calf numbers 9 and 11) or mucopurulent (Calf numbers 10 and 12) consistency. All 4 calves exhibited rapid, shallow breathing by Day 3. Two calves (numbers 10 and 12, developed labored breathing between Days 3 and 9. All 4 calves developed soft, non-formed feces between Days 3 and 11. Two calves (numbers 11 and 12) had severe diarrhea with liquid feces by Day 7. All 4 calves appeared depressed and were inactive by Day 2 to 4. Three of the calves (numbers 10, 11 and 12) were severely depressed from Day 4 to 9. One calf (number 11) was in sternal recumbency and unwilling to stand beginning on Day 6 and continuing until Day 9 when the calf was euthanatized and necropsied.

Control calves (numbers 13 and 14) were clinically normal. Clinical disease was not observed throughout the observation period except for the occurrence of excessive serous nasal discharge from both calves beginning 2 days before the day of viral challenge (Day 2) and continuing until the day of viral challenge (Day 0).

Necropsy

Gross lung lesions were not observed at necropsy in any of the 4 calves in Group 1 (Calf numbers 1, 2, 3 and 4) which had been exposed with BVDV.

Gross lesions were not present in Calf number 9 necropsied 3 days after being dual-inoculated with BRSV/BVDV (group 3). Calf number10 had occasional dark red, firm lung lobules in the cranial lung lobe when necropsied 6 days after dual BRSV/BVDV inoculation. Gross lesions present in lungs of calf number 11 at necropsy 9 days after exposure with BRSV and BVDV consisted of dark red, firm areas involving 80% of the right cranial lung lobe and 25% of the cranial portion of left caudal lung lobes. Calf number 12 had extensive gross lung lesions which were evident at necropsy on Day 12 after exposure with BRSV and BVDV. Caudal lung lobes did not collapse when the thoracic cavity was opened. The distal third of the right middle lung lobe and caudal part of the left cranial lung lobe were dark red and firm. Multiple linear erosions were present in the esophagus. Bilateral laryngeal ulcers covered with fibrinonecrotic debris approximately one centimeter in diameter were located at the opposing margins of the arytenoid cartilages.

Gross lesions associated with viral infections were

not present in either of the 2 control calves in Group 4 (numbers 13 and 14) when necropsied on Days 6 and 12 respectively.

Histopathologic findings

Calf number 1 from group 1, necropsied on Day 3, had multifocal peribronchial and peribronchiolar lymphoid hyperplasia. Calf number 2, necropsied on Day 6, had marked diffuse lymphoid depletion in the thymus. Mild depletion of lymphocytes was seen in lymphoid follicles of the spleen. Multifocal lymphoid necrosis was noted in mesenteric lymph node and Peyer's patch. Calf number 3, necropsied on Day 9 had mild to marked lymphoid necrosis and depletion in thymus, mesenteric lymph node, Peyer's patch, and spleen. Calf number 4 had marked lymphoid depletion of the thymus. Mild lymphoid depletion was seen in Peyer's patch and spleen. Ulceration of the ileal mucosa was noted. Significant lesions were not observed in lung, tracheobronchial lymph node, mesenteric lymph node, liver, kidney, rumen, duodenum, jejunum, and cecum. Bacterial pathogens were not isolated from lung, liver, kidney, or spleen of calf numbers 1, 2, 3 and 4.

Two (numbers 5 and 6) of the 4 Group 2 calves which were inoculated with BRSV only and necropsied on Day 3 and 6, did not have microscopic lesions. Calf numbers 7 and 8 necropsied on Day 9 and 12, respectively, had microscopic evidence of a mild pneumonia. Sections of affected lung from Calf number 7 was characterized by lobular atelectasis, congestion, and edema. Alveoli were filled with macrophages and fewer neutrophils. Numerous neutrophils were present in epithelium of larger airways. There was mild lymphoid depletion of tracheobronchial lymph node and mesenteric lymph node. Microscopic examination of sections of affected lung of Calf number 8, showed lobular atelectasis with bronchioles filled with neutrophils and occasional aggregates of neutrophils in alveoli. Rare syncytia were observed in airway epithelium. Few neutrophils were present in epithelium of bronchi. Bronchial lumens contained plugs of neutrophils. There was moderate multifocal peribronchial and peribronchiolar lymphoid hyperplasia.

Microscopic examination of tissues from Group 3 calves, inoculated with both BRSV and BVDV, showed that Calf number 10, necropsied on Day 6, had microscopic lesions in several tissues. Lungs had lobular atelectasis, congestion, and edema. Multifocal areas of bronchiolar epithelial necrosis were evident. A syncytial cell was present in the lumen of a bronchiole. Numerous neutrophils were present in lumens of larger airways. There was mild lymphoid depletion in the thymus. Few neutrophils were present in tracheobronchial lymph node. Mild to moderate lymphoid necrosis and/or dropout occurred in mesenteric lymph node, Peyer's patch, cecal tonsil, and spleen. Calf number 11, necropsied on Day 9, had lobular atelectasis, congestion, and edema in the lung. Alveoli were filled with macrophages and fewer neutrophils. Numerous neutrophils were present in epithelium of larger airways. There was moderate to marked lymphoid depletion and necrosis in thymus, tracheobronchial lymph node, mesenteric lymph node, Peyer's patch, and cecal tonsil. Calf number 12, necropsied on Day 12, lung sections displayed lobular atelectasis with bronchioles filled with neutrophils and occasional aggregates of neutrophils in alveoli. Rare syncytia were present in airway epithelium. Few neutrophils were present in epithelium of bronchi and bronchial lumens contained plugs of neutrophils. Moderate to marked lymphoid depletion was noted in thymus, mesenteric lymph node, Peyer's patch, cecal tonsil and spleen. Occasional mucosal ulcers were noted in the jejunum. There was focal loss of mucosal crypt epithelium and collapse of mucosal architecture in the ileum. Bacterial pathogens were not isolated from lung, liver, kidney, or spleen of calves in Group 3 (Numbers 9, 10, 11 and 12).

Group 4 calves, (Numbers 13 and 14), the control animals, necropsied on Days 6 and 12, respectively, did not have significant microscopic lesions in tissue sections examined. Bacterial pathogens were not isolated from lung, kidney, or spleen of either noninoculated calf nor from the liver of Calf number 14.

Immunohistochemical findings

BVDV antigen was detected in thymus and Peyer's patch of BVDV-inoculated calf number 3, necropsied on Day 9, and dual BRSV/BVDV-inoculated calves, numbers 11 and 12, necropsied on Day 9 and 12, respectively. Bovine respiratory syncytial virus specific antigen was detected in cytoplasm of airway epithelial cells or cellular exudate of avidin-biotin-stained, sections of BRSV-inoculated Calf number 7, which was necropsied on Day 9; and dual BRSV/BVDV-inoculated Calf number 10, necropsied on Day 6. Immunohistochemical tests on lung tissue sections from the remaining 10 calves were negative for BRSV antigen. Bovine viral diarrhea virus antigen was not detected in lung sections from the 14 calves.

Virologic findings

Bovine respiratory syncytial virus was isolated from nasal swabs of calves inoculated with only BRSV of dual-infected with BRSV/BVDV at the same frequency (onset and duration of shedding). Bovine respiratory syncytial virus was present in nasal secretions between Day 1 and 7, in 7 of the 8 calves inoculated with BRSV or infected with BRSV/BVDV. Bovine respiratory syncytial virus was not reisolated from nasal swabs of 1 calf (number 5). Bovine respiratory syncytial virus was

isolated from lung samples of Calf number 6 which was inoculated with BRSV and necropsied on Day 6, and from Calf numbers 9 and 10, inoculated with BRSV and DVDV and necropsied on Days 3 and 6, respectively. Bovine respiratory syncytial virus was not isolated from lung tissues of the remaining 11 calves nor from the tracheobronchial lymph nodes of any of the 14 calves.

Bovine viral diarrhea virus was isolated from nasal swabs of all calves exposed with BVDV or dual-infected with BRSV/BVDV beginning on Day 2 and continuing until necropsy, with the exception of the 2 calves (Calf numbers 4 and 12) which were necropsied on Day 12. Calf number 4 (BVDV inoculated) and Calf number 12 (dual BRSV/BVDV inoculated) shed BVDV in nasal secretions until Day 11 and 12, respectively. All nasal swabs of calves inoculated with BRSV (numbers 5, 6, 7 and 8) and control calves (numbers 13 and 14) were negative for BVDV.

Bovine viral diarrhea virus was present in blood buffy coat cell preparations from 3 of 4 calves inoculated with BVDV (numbers 2, 3 and 4) and all of the dual BRSV/BVDV calves (numbers 9, 10, 11 and 12) beginning on Day 3 or 4 and continuing until necropsy. All blood cell buffy coat specimens from calves inoculated with BRSV (numbers 5, 6, 7 and 8) and control calves (numbers 13 and 14) were negative for BVDV.

Bovine viral diarrhea virus was isolated from all tissues of the BVDV-inoculated or dual BRSV/BVDV-inoculated calves necropsied on Day 3, 6 and 9. Bovine viral diarrhea virus was isolated from lung of calf number 4 (BVDV-inoculated). Neither BRSV nor BVDV was isolated from post-mortem tissues of the 2 control calves, (numbers 13 and 14), necropsied on Days 6 and 14, respectively.

Discussion

Bovine viral diarrhea virus potentiated BRSV infection of pulmonary airway epithelial cells causing exacerbation of lung injury leading to augmentation of clinical signs of respiratory disease in 205 kg feeder calves in the present study. Clinical signs of BRD (depression, serous to mucopurulent nasal discharge, and dyspnea) were more severe and extent of lung injury was greater in calves inoculated with BRSV and BVDV than in calves inoculated with either virus alone. Magnitude and character of clinical responses of calves to single viral infections in the present study were equivalent to earlier reports of clinical responses of calves of the same age to experimental infections with BVDV[18] or BRSV.[19] All 4 calves infected with BRSV and BVDV developed mild signs of upper respiratory tract infection after inoculation consisting of moderate depression, and serous to mucopurulent nasal discharge. The condition of the dual virus infected calves progressed to signs of lower respiratory tract disease which was manifested by apnea and dyspnea. Clinical condition of the dual virus-infected calves continued to deteriorate daily until they were severely depressed or recumbent. Dual virus-infection did not affect the onset or duration of viral shedding in nasal secretions since both viruses were present from only Day 2 to 7 after viral inoculation in calves infected with either virus alone or in combination. This 7 day duration of BRSV shedding is consistent with BRSV shedding patterns in experimentally-inoculated calves[19] or sheep.[20] Similarly, neither the onset nor duration of BVDV viremia was affected by concurrent BRSV infection since BVDV was usually present in buffy coats from 2 days after inoculation until the animals were euthanatized.

Potentiation of pathogenic effects and clinical signs of BRSV by BVDV during dual BRSV/BVDV infections in calves, resulted in more extensive lesions in the respiratory tract compared to BRSV alone. Dual viral infection induced development of macroscopic pulmonary lesions consisting of dark red firm areas in the cranial lungs. Microscopic lesions consisting of multifocal areas of brochiolar epithelial necrosis with occasional syncytia were associated with BRSV infection in calves inoculated with BRSV alone or in combination with BVDV. Results from immunohistochemical tests showed that brochiolar epithelial necrosis was due to BRSV based on localization of BRSV antigen in the cytoplasm of airway epithelial cells. BRSV was reisolated from lung tissues of calves killed 6 days after being inoculated with BRSV alone or in combination with BVDV and from the calf euthanitized 3 days after inoculation with BRSV and BVDV. The presence of BRSV in postmortem lung tissues corresponded with onset of bronchopneumonia in the calf of the same treatment group which was killed 3 days later.

Increased severity of clinical signs and extension of gross pulmonary lesions in dual virus-infected calves over calves infected with BVDV or BRSV individually, indicated that BVDV potentiated BRSV-induced lung injury. Increased severity and expanded distribution of microscopic lesions consisting of lymphoid depletion in tracheobronchial, mesenteric and cecal lymph nodes of dual virus-infected calves compared to BRSV-infected calves indicated an immunosuppressive role for BVDV which resulted in potentiation of disease. The presence of BVDV antigen in the thymus and Peyer's patch of 3 of the 8 calves inoculated with BVDV was supporting evidence of the negative effects of BVDV on the immune system which culminated in augmented BRSV pulmonary infection. These results led us to conclude that potentiation of BRSV infection by BVDV in the present study was due to the immunosuppressive properties of BVDV. Immunosuppression can be attributed to the lymphotropic properties of BVDV.[6] Bovine virus diar-

rhea virus infection causes decreases in the absolute numbers of B and T lymphocytes in acute infections[7] and decreases in immunoglobulin secretions.[9,11] Neutrophil function is down-regulated[12,13] and bacterial clearance[14] is impaired by BVDV. Collectively, these immunosuppressive properties of BVDV could account for the potentiation of BRSV infection by BVDV observed in the present study. It has been shown that T cell mediated immune response plays a role in protection against BRSV.[21] Decreases in T cell numbers as a result of BVDV infection could be a specific cause for enhanced disease due to dual viral infection.

An apparent potentiation of BVDV infection of the digestive tract by BRSV also occurred and was manifested by intensification of enteric clinical signs in calves dually-infected with BRSV and BVDV compared to calves infected with only BVDV. This is supported by the fact dual virus-infected calves were moderately depressed and developed non-formed feces by Day 3 which progressed to severe watery diarrhea on Day 7. Calves inoculated with BVDV, in contrast, became only moderately-depressed and diarrheic on Day 3 and remained in that condition until necropsy. Only 2 of the 4 calves inoculated with BRSV developed mild depression (without diarrhea) which was evident for one day. BVDV's role in causing moderate diarrhea in BVDV-infected calves and severe diarrhea in the dual BRSV/BVDV-infected calves was confirmed by localization of BVDV-specific antigen. Antigen was present in cells in areas of multifocal lymphoid necrosis within mesenteric lymph nodes and Peyer's patches virus isolation from the affected organs also confirmed the presence of BVDV.

Potentiation of BRSV infection and pulmonary injury by BVDV supports the generally accepted hypothesis for the pathogenesis of classical, naturally-occurring, clinically-severe BRD which is typically due to mixed viral and bacterial infections. This study supports findings of a serologic study in which BVDV was associated with initiation of BRD and it was concluded that multiple viruses concomitantly infect animals and could be important in pathogenesis of naturally-occurring pneumonia in feeder cattle.[22] Our results indicate that BRSV and BVDV, together, are important causes of naturally-occurring BRD. This conclusion is further supported by the serological study of feedlot cattle in Canada in which BRSV, BVDV and *Pasteurella spp.* were associated with 60% of BRD occurrence.[23]

Acknowledgments

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Table 1—Criteria for postchallenge clinical scoring

NASAL DISCHARGE	RESPIRATORY RATE/CHARACTER	CONSISTENCY OF FECES	APPETITE	CONDITION
0 = normal	0 = normal	0 = normal	0 = normal	0 = normal
1 = excessive serous	1 = rapid, shallow	1 = soft, non-forming	1 = anorexic	1 = depressed, inactive
2 = copious mucopurulent	2 = rapid, shallow, occasional cough	2 = watery, semi-solid	2 = dehydrated	2 = severely depressed
	3 = labored, dyspneic, cough	3 = liquid		3 = downer

Table 2—Necropsy schedule

TRIAL DAYS 3 & 9	TRIAL DAYS 6 & 12
1 BRSV-challenged calf	1 BRSV-challenged calf
1 BVDV-challenged calf	1 BVDV-challenged calf
1 BRSV-BVDV–challenged calf	1 BRSV-BVDV–challenged calf
	1 control calf

Table 3—Responses in calves challenged with BRSV and BVDV

CHALLENGE GROUP (n)	CLINICAL RESPONSE	GROSS LESIONS	MICROSCOPIC LESIONS	IMMUNOHISTOCHEMISTRY	VIRUS ISOLATION
I CONTROLS (2)	Normal	No lesions associated with viral infection	No significant lesions	Neither BRSV nor BVDV antigen	Neg. for BRSV Neg. for BVDV
II BRSV CHALLENGE (4)	Serous nasal discharge Rapid, shallow breathing Depression Inactivity Occasional cough	Red, firm lobules in cranial lung lobes of 2 calves	Lobular atelectasis Congestion Edema Alveoli filled with macrophages Neutrophils in epithelium of large airways Rare syncytia Mild lymphoid depletion of tracheobronchial lymph node and mesenteric lymph node	BRSV specific antigen in cytoplasm of airway epithelial cells or airway cellular exudate of lungs	BRSV isolated from nasal swabs and nasal secretion; also from lung tissue of 1 calf
III BVDV CHALLENGE (4)	Excessive serous nasal discharge Rapid, shallow breathing Abnormal fecal consistency Anorexia Generalized depression Elevated temperature	No gross lung lesions	Multifocal peribronchial and peribronchiolar lymphoid hyperplasia in lung sections Eosinophils in Peyer's patch Marked diffuse lymphoid depletion in thymus Mild to marked depletion of lymphocytes in lymphoid follicles in spleen Multifocal lymphoid necrosis in mesenteric lymph node and Peyer's patch	BVDV antigen detected in thymus and Peyer's patch	BVDV isolated from nasal swabs, nasal secretions, and blood buffy coat
IV BRSV-BVDV CHALLENGE (4)	Excessive serous or mucopurulent nasal discharge Rapid, shallow breathing Soft, unformed feces Severe diarrhea Depression Inactivity Sternal recumbency	Red, firm lobules in cranial lung lobe (up to 80% of right lobe; 25% of left caudal lobe) Caudal lung lobes did not collapse when thoracic cavity was entered Multiple linear erosions in esophagus Bilateral laryngeal ulcers covered with fibrinonecrotic debris at opposing margins of arytenoid cartilage	Lobular atelectasis Congestion Edema Multifocal areas of bronchiolar epithelial necrosis Syncytial cell in lumen of bronchiole Neutrophils in lumens of large airways Mild lymphoid depletion in thymus Neutrophils in tracheobronchial lymph node Mild to moderate lymphoid necrosis and/or depression in mesenteric lymph node, Peyer's patch, cecal tonsil, spleen Alveoli filled with macrophages Intraepithelial abscess in rumen Occasional mucosal ulcer in jejunum Focal loss of mucosal crypt epithelium and collapse of mucosal architecture in ileum Occasional aggregates of neutrophils in alveoli	BRSV-specific antigen in cytoplasm of airway epithelial cells BVDV antigen in thymus and Peyer's patch	BRSV isolated from nasal swabs and nasal secretions; also from lung tissue of 2 calves BVDV isolated from nasal swabs, nasal secretions, and blood buffy coat

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Diagnosis of Bovine Respiratory Syncytial Virus Infection: Evaluation of an Enzyme Immunoassay (TESTPACK ABBOTT) and Comparison of Two Sampling Sites

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This study had two objectives: (1) Comparison of two virological methods (enzyme immunoassay - Testpack Abbott - (TP) versus indirect immunofluorenscence (IIF) on Broncho Alveolar Lavage Fluid (BALF). Comparison of sampling from the upper respiratory tract (nasopharyngeal swab - NPS) and lower respiratory tract (BALF obtained by endobronchial endoscopy).

This work involved 83 beef calves, under three months old, from 22 herds. Three to five animals with acute respiratory signs were sampled in each herd.

BVDV was identified by IIF or TP on BALF in 5 of the 22 herds (22.7%) and in 21 of the 83 calves (25.3%). When Indirect Immunofluorescence was the gold standard, the sensitivity and specificity of TESTPACK ABBOTT were 95% (19/20) and 98.4% (62/63) respectively.

Test sensitivity, on an individual basis, was increased by sampling the lower, rather than upper, respiratory tract. Fifteen of the 20 positive results obtained by TP on the BALF were also positive by TP on NPS. All the animals found negative by TP on BALF - sampling were also negative using NPS samples. At least one calf was positive with TP on NPS in the 5 positive herds.

TP is a useful and quick (20 minutes) diagnostic method for field practitioners being easy to perform and easy to interpret.

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