Bovine Respiratory Syncytial Virus Infection in McNay Farm Calves

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Summary

Clinical respiratory disease occurs almost every year in fall calves in the McNay Farm herd. Diagnostic procedures have implicated *Haemophilus somnus* (*H. somnus*) and bovine respiratory syncyial virus (BRSV) as the infectious agents primarily associated with this disease. Therefore, the 1995 calves were closely monitored after weaning and during the course of a respiratory disease. Serologic evidence indicated the involvement of the same two agents in the pathogenesis of the disease. Also, experimental evidence suggested a role for a preexisting immediate hypersensitivity to *H. somnus* and the development of this type of response to BRSV. We theorize that the pathogenesis of the clinical disease involved infection with *H. somnus*, establishment of immediate hypersensitivity in the lungs, viral infection with associated pathologic lesions, and viral exacerbation of the immediate hypersensitivity reaction with resultant clinical signs and tissue damage.

Introduction

Calves in the McNay Farm herd have been monitored for respiratory disease for several years by observation of clinical signs, necropsies and serologic methods. Widely recognized pathogens including bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis virus, parainfluenza type 3 virus, adenovirus type 3, bovine viral diarrhea virus, *Haemophilus somnus (H. somnus)*, *Pasteurella haemolytica* (*P. haemolytica*) and *Pasteurella multocida* are present in this herd. The most serious respiratory disease problem has been in fall calves that annually break with clinical disease in December or early January at 90 to 130 days of age. Morbidity is high but mortality has been reduced from 20% several years ago to 0 to 5% in recent years due in part to improved management practices. *Haemophilus somnus* was a major contributor to mortality in the 1980s but in recent years, although the organism is still present in the herd, has not induced the severe bacterial pneumonia with associated mortality. Rather, the clinical disease is an acute pneumonia with the involvement of *H. somnus* and BRSV.

These fall calves acquire appreciable levels of maternal antibodies from their dams that apparently protect the calves from BRSV infection during the neonatal period. Previous attempts to immunize calves in October or November with commercial modified-live (MLV) BRSV vaccine have not prevented clinical disease. Maternal antibodies are at a level that apparently blocks an active immune response to the vaccine. We report observations on 1995 fall calves that developed an acute episode of respiratory disease shortly after vaccination with a modified live BRSV vaccine. A group of these calves had received a primary dose of an experimental BRSV subunit vaccine.

Materials and Methods

Eighty-seven calves were weaned on November 15, 1995, and randomly distributed into four pens. Pens one and two as well as three and four were separated only by a fence, but there was a 12-foot wide alley between the two pen units. On November 21 all calves were bled for serum, and 20 calves (10 each in pens one and two) were administered an experimental BRSV subunit vaccine. On December 6 calves in pens one, two and three were bled and calves in pens one and two administered a two ml dose of a commercial modified-live BRSV vaccine. At this time a dry cough was noted among calves in all pens.

Our previous experience with fall calves suggested that clinical observation should be supplemented with other determinations including skin testing and serology. Therefore, sequential blood samples were collected and serum was stored at –20°C. Serologic tests included virus neutralization tests for BRSV antibody and ELISA for antibodies to *P. haemolytica* and *H. somnus*. The ELISA procedure for IgG antibodies utilized a peroxidase-labeled anti-bovine IgG secondary antibody. The ELISA procedure for IgE antibodies utilized a mouse monoclonal anti-bovine IgE antibody preparation and peroxidase labeled anti-mouse IgG. Two positive and negative sera were included in each microtiter plate at the same dilutions as the test sera. This permitted calculations and reporting of the level of antibodies as a percentage of the standard:

$$\text{Percentage of Standard} = \frac{\text{O.D. Test} - \text{O.D. Negative}}{\text{O.D. Standard} - \text{O.D. Negative}}$$

Skin tests were conducted by the injection of 100µl of antigen intradermally on the inside of the thigh. The *H. somnus* antigen was supernatant from washed *H. somnus* organisms passed through a French press and centrifuged for 30 minutes at 5000g. The BRSV antigen was culture supernatant from BRSV infected bovine lung cells. The antigen preparation was heated at 56°C for 30 minutes to inactivate the virus. The experimental protocol was as follows:

11/15/95	Calves Weaned
11/21/95	Calves Bled
	Vaccinated 20 Calves -- Pens 1 and 2
12/06/95	Calves Bled -- Pens 1, 2, and 3

	Vaccinated (MLV Vaccine) Pens 1 and 2
12/12/95	Calves Bled -- Pens 1 and 2
	Skin Tested 4 Calves -- *H. somnus*
12/13/95	Sick Calves -- Pen 4
	Temperatured Calves -- Pens 3 and 4
	Switched Calves -- Pens 2 and 3
12/14/95	One Calf Dead -- Pen 4
	Temperatured Calves -- Pens 1, 2, and 3
12/15/95	Bled Calves -- Pens 1 and 2
	Temperatured Calves -- Pens 1, 2, and 3
	Skin Tested 6 Calves -- BRSV and *H. somnus*
	Nasal Swabs -- 10 Calves
	Second Calf Dead
12/16/95	Temperatured Calves -- Pens 1, 2, and 3
12/18/95	Temperatured Calves -- Pens 1, 2, and 3
12/20/95	Bled Calves -- Pens 1, 2, and 3
01/10/96	Bled Calves -- All Pens

Observations

Signs of clinical respiratory disease appeared in calves in pen four (nonvaccinated) on December 13 (high temperatures, depression and labored breathing). At this time many calves in pen three had an increased rectal temperature but did not show clinical signs. On December 14 overt clinical disease was observed in calves in all pens, and many calves had increased rectal temperatures up to 106.1°F. One calf in pen four died and necropsy revealed extensive consolidation and emphysema in the lungs. Microscopically, there was severe proliferative and necrotizing interstitial pneumonia and bronchiolitis. There was also moderate suppurative bronchopneumonia. Presence of BRSV was demonstrated by direct fluorescent antibody procedures, but no bacteria were isolated from lung tissue. A second calf died on December 15, and necropsy revealed similar lesions in the lungs. Many calves were treated with antihistamines and antibiotics, and the remaining calves returned to normal by December 20.

Interest in immediate hypersensitivity as a contributor to respiratory disease led us to skin test four animals on December 12. Three of these animals had positive reactions to *H. somnus* antigen. Skin tests on six animals on December 15 were positive for both *H. somnus* and BRSV antigens. Two of these animals developed systemic anaphylactic shock within 10-15 minutes, were treated with epinephrine and antihistamine, and recovered.

Serologic tests served to confirm the involvement of infectious agents in this disease. Neutralizing antibody titers for BRSV were variable among calves at the start of the experiment with most calves possessing moderate titers (probably of maternal origin). The titers of BRSV antibodies increased in most calves by December 20 (Table 1), but the increase was moderate and titers were declining by January 10. Antibody titers of animals given the experimental vaccine were not different from the controls. Also the vaccine did not protect the calves from clinical disease although disease appeared to be less severe, and no death losses occurred in that group. Serum antibody levels to *H. somnus* of a few calves prior to the outbreak of clinical disease are provided in Table 2. Levels of IgG class antibodies were moderate and did not increase during the period of observation. However, appreciable levels of IgE class antibodies were present in the serum of these calves prior to the development of clinical disease. Calves had low titers of antibodies to *P. haemolytica* at the start with a marked increase by December 20 (data not included).

Table 1. BRSV antibody titers of selected calves.

	Date			
Calf No.	11/21	12/6	12/15	12/20
000	16	8	8	256
002	128	64	64	128
006	16	8	4	16
178	32	16	8	256
180	32	32	16	16
194	16	4	4	64
326	2	4	32	256

Table 2. *Haemophilus somnus* antibody levels in selected calves.

	Date					
	11/21		11/29		12/6	
Calf No.	IgG	IgE	IgG	IgE	IgG	IgE
000	16.3*	63.3	7.8	39.8	9.4	42.7
002	16.8	54.5	7.8	44.2	3.8	38.0
006	15.7	40.2	7.7	33.8	6.1	43.4
008	20.5	9.0	18.3	10.5	6.8	19.9
010	15.4	64.8	15.8	57.1	16.6	60.0

*Percentage of standard

Conclusions

Clinical respiratory disease caused by infection with BRSV developed in calves that had residual maternal antibodies. This was particularly interesting because even a low level of maternal antibodies precludes successful immunization with modified-live BRSV vaccine. Consequently, immunization of calves against BRSV prior to natural exposure to the virus remains a major problem. A particularly important finding of this experimentation comes from the clinical and laboratory observations. Many of the calves were apparently infected with *H. somnus* and had developed appreciable levels of circulating IgE class antibodies to that organism. This is consistent with the positive skin test reactions induced in the calves. These finding are supportive for the presence of an immediate type of hypersensitivity in these calves. This preexisting hypersensitivity probably contributed to the severity of the clinical disease. Although BRSV infection can induce the production of IgE class antibodies and an immediate hypersensitivity, the preexisting hypersensitive state

probably enhanced the response. The severity of the clinical disease may result from the combination of viral infection and products of the immediate hypersensitivity reaction. Viral infection and Type I interferon have been reported to accentuate release of mediators of allergic reactions, and BRSV may have this capability. Therefore, it is reasonable to conclude that expression of clinical disease resulted not only from cell destruction by the virus but an immediate hypersensitivity reaction in the lungs.

The failure of the experimental vaccine to protect the calves could have been expected under the circumstances. The vaccine is a subunit intended only to prime the animal for secondary responses to the virus. The secondary response would be induced by the second vaccination with the whole virus commercial vaccine. However, natural infection with wild strain, virulent virus interceded shortly after the booster vaccination. Thus, there was inadequate time for development of protective immunity.

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