ARTICLES

Association of Bovine Viral Diarrhea Virus with Multiple Viral Infections in Bovine Respiratory Disease Outbreaks

Lisette Richer, Paul Marois and Lucie Lamontagne

Abstract

We investigated eleven outbreaks of naturally occurring bovine respiratory diseases in calves and adult animals in the St-Hyacinthe area of Quebec. Specific antibodies to bovine herpesvirus-1, bovine viral diarrhea virus, respiratory syncytial virus, parainfluenza type 3 virus, reovirus type 3, and serotypes 1 to 7 of bovine adenovirus were found in paired sera from diseased animals. Several bovine viruses with respiratory tropism were involved concomitantly in herds during an outbreak of bovine respiratory disease. In addition, concomitant fourfold rises of antibody titers were frequently observed to two or more viral agents in seroconverted calves (61%) or adult animals (38%). Bovine viral diarrhea virus was found to be the most frequent viral agent associated with multiple viral infection in calves only (92%).

Résumé

L'association du virus de la diarrhée bovine avec des infections virales multiples dans des élevages de bovins atteints de maladies respiratoires

Les auteurs ont étudié 11 élevages bovins de la région de St-Hyacinthe au Québec où des maladies respiratoires s'étaient déclarées. Des anticorps spécifiques aux virus herpes bovin de type 1, de la diarrhée virale bovine, du respiratoire syncytial, du parainfluenza de type 3, du réovirus de type 3 et des sérotypes 1 à 7 de l'adénovirus bovin ont été trouvés dans les sérums pairés des animaux malades. L'analyse des séroconversions observées vis-à-vis ces virus ont montré que plusieurs virus bovins étaient impliqués de façon concomitante dans l'élevage lors de l'apparition de la maladie respiratoire. De plus, des séroconversions vis-à-vis 2 virus ou plus ont été fréquemment rencontrées chez les veaux (61%) ou chez les adultes (38%). Le virus de la diarrhée virale bovine a été le virus le plus souvent associé à ces infections virales multiples chez les veaux (92%).

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Centre de recherche en médecine comparée, Institut Armand-Frappier, Université du Québec, 531, boul des Prairies, Laval, Québec H7N 4Z3 (Richer, Marois) and Département des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888, Succ A, Montréal, Québec H3C 3P8 (Lamontagne).

Reprint requests to Dr. L. Lamontagne.

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Introduction

Bovine respiratory diseases (BRD) are significant to the economics of the cattle industry. The generally accepted hypothesis for the pathogenesis of BRD involves a sequential cascade of events initiated by stress, predisposing animals to viral infections, which in turn facilitate rapid bacterial invasion. Various viruses have been associated with BRD such as: bovine herpesvirus 1 (BHV1), parainfluenza type 3 (PI3), bovine respiratory syncytial (BRS) virus, several serotypes of reoviruses and bovine adenoviruses (BA) (1). In addition, bovine viral diarrhea (BVD) virus has occasionally been encountered in BRD outbreaks, usually in association with other pathogens, in spite of its well-recognized enterotropism and lymphotropism (2-6). Experimental evidence to support the involvement of BVD virus in BRD has been disappointing (7-9). However, some pneumopathogenic BVD viral strains that induced only a mild clinical respiratory disease in calves, exacerbated the disease when associated with *Pasteurella haemolytica* (10,11). Therefore, it is reasonable to expect that synergism may occur between BVD virus and pathogenic bovine viruses with respiratory tropism, causing a more severe disease.

There are only a few reports on the occurrence of concomitant seroconversions to more than one virus during BRD outbreaks, either with or without association with BVD. Rosenquist *et al* (12) have previously reported dual (BVD and PI3) and multiple (BVD, PI3 and rhinovirus) viral infections in diseased calves as evidenced by virus isolation or by a fourfold increase of specific viral antibodies. A dual viral infection in one calf involving BA3 and BRS viruses has also been reported (13). No other reports are available on the

TABLE 1
Antibodies to Bovine Viruses with Respiratory Tropism in Paired Sera Collected from Calves and Adults during Respiratory Disease Outbreaks

Animals	Serological Status	Number of Animals with Antibodies to Bovine Viruses with Respiratory Tropism in Paired Samples (%)											
		BHV1	BVD	PI3	REO3	BRS	BA1	BA2	BA3	BA4	BA5	BA6	BA7
Calves	Seroconversion[a]	3(11)	16(57)	0	1(4)	7(31)	1(4)	1(3)	4(18)	2(8)	1(4)	1(4)	2(8)
	Seropositive[b]	6(21)	8(29)	25(89)	12(39)	7(30)	15(60)	7(25)	4(18)	11(42)	8(32)	16(64)	6(24)
	Seronegative[b]	19(68)	4(14)	3(11)	15(57)	9(39)	9(36)	20(71)	14(64)	13(50)	16(64)	8(32)	17(68)
Adults	Seroconversion	1(3)	1(3)	0	2(6)	5(18)	6(11)	2(6)	2(6)	N[c]	N	N	N
	Seropositive	23(68)	19(54)	30(91)	10(32)	10(34)	28(80)	25(80)	18(54)	N	N	N	N
	Seronegative	11(31)	15(43)	3(9)	19(62)	13(48)	3(9)	5(15)	13(39)	N	N	N	N

[a]Fourfold rise of antibody titers in paired samples collected 3 weeks apart
[b]Seropositive defined as ≥10 for PI3 and RE03; ≥4 for BHV1 or ≥8 for other viruses, and below these thresholds, as seronegative in acute and convalescent sera
[c]N = not determined

importance of multiple viral infection in BRD outbreaks in cattle herds.

The purpose of the present study was to investigate in diseased calves and adult animals the involvement of multiple viral infections in outbreaks of BRD.

Materials and Methods

Outbreaks

Eleven outbreaks of BRD were investigated in calves and adult animals in the St-Hyacinthe area of Quebec. Herds were separated into two groups: group 1 consisted of herds A to E in which only calves had clinical signs of acute BRD, and group 2, herds F to K, in which only adult animals were diseased. All herds under study had animals with clinical signs of acute respiratory disease for less than ten days, and these signs consisted of nasal discharge, cough, dyspnea, and in some herds, conjunctivitis or abortions. All herds selected were Holsteins except for herd A, which consisted of Herefords. Diseased animals showing respiratory signs, representing about 10% of the total number of animals in each herd, were selected for serological tests.

Cell cultures

Primary bovine fetal skin, testis, kidney and lung cells were prepared in Eagle's minimal essential medium containing 10% gamma-globulin free fetal calf sera (Flow Laboratories, McLean, Virginia) and antibiotics, and incubated under 5% CO_2 in a humidified atmosphere. Cell cultures were checked for freedom from contaminating latent viral or mycoplasmal agents, using standard procedures.

Viruses

Bovine herpesvirus 1 virus, Colorado strain; BVD-MD virus, NADL strain; PI3, SF4 strain; and serotypes 1 to 7 of bovine adenoviruses were obtained from the American Type Culture Collection (Bethesda, Maryland). BRS and Reovirus-3 (Reo3) were obtained from Microbiological Associates (Bethesda, Maryland). All strains of viruses were cultured in bovine testis cell cultures except PI3, which was cultured in bovine primary kidney or fetal lung cells. Secondary cell cultures were infected at a multiplicity of infection levels of 0.01 to 0.1 with BHV1, BVD, PI3, and various serotypes of BA viruses. Cells were harvested when typical cytopathic effects reached 50%–75% of the cell monolayer. Viral suspensions were clarified by centrifugation (10,000 × *g* for 30 min) after three freezing-thawing cycles and were then titrated. Viral suspensions were separated in aliquots and stored at −70°C until used.

Serology

Paired sera were obtained at three week intervals and stored at −20°C before being tested. All sera were heat-inactivated at 56°C for 30 min. Antibody titers to BHV1, BVD, and BA serotypes 1 to 7 were determined by seroneutralization tests in secondary fetal bovine testis or skin cells by using 100 median tissue culture infectious dose ($TCID_{50}$). Hemagglutination-inhibition assays were used to evaluate antibody titers to PI3 and Reo3 viruses using 0.25% bovine or human O red blood cells, respectively, and four units of antigen. Antibodies to BRS virus were detected by a modified complement fixation test (14) by using eight units of RS virus antigen, two hemolytic units of guinea pig complement, and 2% sheep red blood cells. Mean antibody titers were expressed as the last positive reciprocal dilution. All serological tests were performed in triplicate.

Acute and convalescent sera with antibody titers ≥10 for PI3 and Reo3, ≥4 for BHV1, or ≥8 for other viruses were recorded as seropositive and, below these thresholds, as seronegative. Seroconversion was interpreted as a fourfold rise of antibody titers between the first and second sera.

Results

Serological Findings during BRD Outbreaks

The majority of calves seroconverted to BVD in contrast with only one adult animal (Table 1). Seroconversions to BRS virus were observed in several calves and adult animals. Some seroconversions to other viruses were detected. Seropositivity to PI3 was frequently observed in calves and adult animals. Antibodies to various serotypes of BA viruses were detected at low titers in the majority of calves (BA1 and BA6), and in adult animals (BA1, BA2 and BA3). No antibody to BHV1, Reo3, and other serotypes of BA viruses was

TABLE 2
Number of Animals Seroconverting to Viruses with Respiratory Tropism

	No. of Seroconversions in Herds									
	Calves					Adult Animals				
Virus	A	B	C	D	E	F	G	H	I	J	K
BHV 1	1	1	0	1	0	0	1	0	0	0	0
BVD-MD	7	0	5	3	0	0	0	0	1	0	0
PI3	0	0	0	0	0	0	0	0	0	0	0
REO3	0	0	1	0	0	1	1	0	0	0	0
BRS	4[a]	0	3	0	0	3	0	2	0	0	0
BA1	0	0	1	0	0	2	1	0	2	1	0
BA2	1	0	0	0	0	1	0	0	0	0	1
BA3	3	0	1[a]	0	0	0	1	0	0	0	1
BA4	0	0	1	0	0	N	N	N	N	N	N
BA5	0	0	2	0	0	N	N	N	N	N	N
BA6	0	0	0	1	0	N	N	N	N	N	N
BA7	0	0	1	0	1	N	N	N	N	N	N
Total number of tested animals	8	4	9	3	4	6	5	4	5	8	10

[a]Only six calves were tested for this antibody
N = not determined

found in the majority of calves; adult animals were usually seronegative to these viruses.

Concomitant Seroconversions to Bovine Viruses with Respiratory Tropism

Seroconversions to from one to five different viral agents were encountered in herds A to E (Table 2). In addition, the number of seroconversions to different viruses was particularly high in herd C in which seroconversions to BA4, BA5 and BA7 viruses were also detected. Seroconversions to BVD virus were observed in at least half of calves in herds A, C and D. In addition, some calves of herd A also seroconverted to BRS virus (80%), whereas no predominant seroconversion was observed to a particular virus in herd C. Seroconversion to one to four different viruses, however, was associated with BRD in herds F to K, although we did not check for antibodies to BA4 to BA7 viruses; BRS viral seroconversion was frequent in herds F and H only.

Concomitant Multiple Seroconversions in Calves and Adult Animals

Seroconversions to various viruses were found in 71% of calves and 37% of adult animals (Table 3). Sixty-five percent of calves and 38% of adult animals which showed seroconversions were concomitantly infected by two or three viral agents. Although BHV1, BRS, Reo3, and most of the serotypes of BA viruses were involved in concomitant multiple viral infections, BVD virus was found to be the viral agent most frequently associated with multiple seroconversion in calves (92%). In addition, high antibody levels to this virus were also observed in one calf seroconverting to two other viruses; BVD seroconversion was detected only once in multiple viral infections in adult animals.

Discussion

This work shows that concomitant multiple viral infections are encountered in outbreaks of BRD in cattle herds as indicated by concomitant seroconversions to two or more different viral agents in diseased animals. Serological evidence or isolation of one to three different viral agents in a herd during a BRD outbreak have frequently been observed, but infection with two or three different viruses in one diseased animal has less often been described (8,9,15,16,17). Seroconversions to two or more viral agents in 46% of diseased calves and in some adult animals suggests that several viruses may concomitantly infect an animal. Various associations of bovine viruses with respiratory tropism were identified in concomitant multiple viral infections, such as BHV1, BVD, BRS, Reo3, and various serotypes of BA viruses. However, no seroconversions to PI3 virus were detected in the samples from calves in spite of the well-known role

TABLE 3
Associations of Bovine Viruses with Respiratory Tropism Involved in Multiple Viral Infections in Calves and Adult Animals as Determined by Concomitant Seroconversions

No. of Seroconversions to Concomitant Viral Infections	Calves		Adult Animals	
	Viruses	No. of Animals	Viruses	No. of Animals
2	BVD-MD, BRS	4	REO3, BRS	1
	BVD-MD, BA3	1	REO3, BA3	1
	BVD-MD, REO3	1	BHV1, BA1	1
	BRS, BA1	1[a]	BVD-MD, BA1	1
			BA1, BA2	1
3	BVD-MD, BHV1, BRS	1		
	BVD-MD, BHV1, BA6	1		
	BVD-MD, BRS, BA3	1		
	BVD-MD, BA1, BA3	1		
	BVD-MD, BA2, BA3	1		
	BVD-MD, BA4, BA5	1		
Total number of animals tested		28		35

[a]High antibody titers to BVD were observed in this animal

of PI3 in calf pneumonia (1,7,9). This may have been due to persistence of maternal antibody as demonstrated by low titers in the majority of animals. Dual infections in calves have already been described in a few cases and consisted of an association between BVD and PI3 viruses (12), BVD, PI3 and bovine rhinovirus (9), combinations of BRS, BHV1, BVD, PI3, or BA3 viruses (16,17). Our results are in agreement with these previous observations and show that multiple viral infection is more frequent in calves than previously observed and could be important in the BRD process.

This work also shows that multiple viral infections occurred frequently in association with BVD viral infection in diseased calves suggesting that BVD virus is present in the herd and could be involved in the initiation process of BRD. Immunosuppressive properties of BVD virus could potentiate the pathogenic effects of concomitantly infecting BHV1 virus in cattle. Potgieter *et al* (18) have studied the *in vivo* effects of simultaneous BVD and BHV1 viral infections in calves, and showed that initial BVD virus infection impaired the ability of calves to clear subsequent BHV1 viral infection in the lungs and to contain the latter virus at the infection site.

Bovine viral diarrhea viral infection is ubiquitous in the cattle population. The usual form of infection is subclinical but acute or chronic diseases associated with viral persistence may occur. The various immunosuppressive properties of BVD virus, related to its lymphotropism, have already been described (19-26). This virus can decrease the absolute numbers of B and T lymphocytes and the percentage of T lymphocytes in acutely infected animals (19), or affect the immune response in persistently infected cattle as demonstrated by hyporesponsiveness of lymphocytes to various mitogens (20,21), and decreases in the immunoglobulin secretion by B lymphocytes (22), and in antibody production (5). Impairment of monocyte chemotaxis (22), polymorphonuclear leukocyte function (24), bacterial clearance from blood (25), and release of immunosuppressive substances from infected cells (26) have been also associated with BVD infection. Pneumopathogenicity of BVD-MD virus strains may be related to their ability to impair the pulmonary clearance mechanism, since a cytopathic strain impaired the clearance of *P. haemolytica* more severely than a noncytopathic strain (8,11). Pulmonary clearance is an important barrier to viral infection. Cattle concomitantly infected with BVD and other viruses with respiratory tropism may develop a more severe clinical disease if the viral clearance mechanism is affected by viral replication (18).

Multiple viral infections in an animal may explain severe or recurrent BRD or the apparent lack of protection following a preventive vaccination program including use of BVD live vaccine. The preventive approach of BRD, particularly in calves, should be enlarged to other viruses with respiratory tropism such as RSV or the different serotypes of BA viruses. Further work is in progress to study the impairment of cellular and humoral immune response mechanisms following a BVD viral infection.

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Abstract

Immune Responses to *Mycoplasma bovis* Vaccination and Experimental Infection in the Bovine Mammary Gland

J.T. Boothby, C.E. Schore, D.E. Jasper, B.I. Osburn and C.B. Thomas

This study characterized the immune responses in four vaccinated and four control cows in response to vaccination and experimental intramammary inoculation with *Mycoplasma bovis*. Specific antibody responses occurred in serum and milk in response to vaccination and experimental infection. Lymphocytes from peripheral blood, but not from the mammary gland of vaccinated cows had increased responsiveness to mitogens. No lymphocytes tested were responsive to *M. bovis* antigen. Both vaccination and experimental infection resulted in skin test reactivity. These results imply that vaccination results in immune responses which may alter the course of experimental *M. bovis* mastitis, but may contribute to cellular inflammation.

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