

INFECTIOUS BOVINE RINOTRACHEITIS ASSOCIATED WITH BOVINE VIRAL DIARRHEA AND ENZOOTIC BOVINE LEUKOSIS

HERPESVIRUS BOVINO ASSOCIADO À DIARRÉIA VIRAL BOVINA E À LEUCOSE ENZOÓTICA BOVINA¹

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SUMMARY

This research verified the variation of the occurrence of herpesvirus bovine type 1 (BoHV-1) by its association with two viral infections: bovine viral diarrhea (BVD) and enzootic bovine leukosis (EBL). The following methodologies were used: virus neutralization test (VNT) for both BoHV-1 and BVD diagnosis, and agar gel immunodiffusion (IDGA) for EBL. Five cattle herds were selected in the states of São Paulo and Minas Gerais, three of them were dairy cattle herds, one beef cattle and one crossbred. Every herd had a number of animals seropositive for BoHV-1. From 278 analyzed samples, 54.68% (152/278) were responsive to BoHV-1, 69.70% (194/278) to BVDV-1, and 34.33% (97/278) to EBLV. The statistical analysis showed significant difference ($\alpha = 0.01$) in infection occurrence according to the type of herd and animal age. Dairy cattle were more sensitive to BoHV-1 (81.31%) and to EBLV (49.53%), while in beef cattle the most frequent was BVDV-1 (94.74%). Age was a risk factor only for BoHV-1 and EBLV, the results showed that the older animals were more susceptible. The association analysis also indicated that among BVDV-1 and/or EBLV infected herds the probability of finding BoHV-1 is higher than among herds where these two infections do not occur.

KEY-WORDS: BoHV-1. BVD. EBL. Immunosuppression. Seroprevalence. Viral co-infection in bovine herds.

RESUMO

Esta pesquisa verificou a variação da ocorrência do herpesvírus bovino tipo 1 (BoHV-1) pela associação com duas doenças virais: diarreia viral bovina (BVD) e leucose enzoótica bovina (LEB). Como metodologias foram utilizados o teste de vírusneutralização para detecção de BoHV-1 e BVD, e imunodifusão em gel de ágar para LEB. Foram selecionados cinco rebanhos bovinos, de propriedades localizadas em municípios dos Estados de São Paulo e Minas Gerais, sendo três de exploração leiteira, um de corte e um misto, todos com parte dos animais soropositivos ao BoHV-1. Das 278 amostras analisadas, 54,68% (152/278) foram positivas ao BoHV-1, 69,70% (194/278) ao BVDV-1 e 34,33% (97/278) ao VLEB. Na análise estatística, ao relacionar cada enfermidade com o tipo de exploração do rebanho e a idade dos animais, houve diferença significativa ($\alpha=0,01$). Os rebanhos leiteiros foram mais suscetíveis ao BoHV-1 (81,31%) e a LEB (49,53%), enquanto o gado de corte teve como maior ocorrência o BVDV-1 (94,74%). A idade foi fator de risco apenas para o BoHV-1 e a LEB, sendo os animais mais velhos os mais suscetíveis. As análises associativas também indicaram que em rebanhos infectados por BVDV-1 e/ou LEB, a probabilidade de se encontrar o BoHV-1 é maior do que nas isentas dessas duas enfermidades.

PALAVRAS-CHAVE: BoHV-1. BVD. Co-infecção viral em rebanhos bovinos. Imunossupressão. LEB. Soroprevalência.

¹Research funded by CAPES and FAPESP (Process 06/55899-4)

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INTRODUCTION

The bovine herpesvirus 1 (BoHV-1) belongs to the family of *Herpesviridae* (FENNER, 1987), genus *Varicellovirus*. It is also known as infectious bovine rhinotracheitis virus/ bovine pustular vulvovaginitis (IBR/IPV) (DEL FAVA et al., 2002).

The most important feature of the BoHV-1 is the development of the state of latency (ENGELS & ACKERMANN, 1996). The virus can be reactivated when the animals are exposed to stressful situations that decrease the immune resistance, such as glucocorticoid treatment, parturition, transportation and several other diseases (LEMAIRE et al., 1994).

The most important risk factor associated with the spreading of the disease is the introduction of animals infected with the virus in the herd (VAN SCHAİK et al., 2002). Research shows that older animals are more susceptible to infection, males are also more seropositive compared to females, the denser the herd the more spread becomes the disease and cattle participation in auctions and agricultural fairs are all risk factors (DIAS et al., 2008; BOELAERT et al.; 2005, BARBOSA et al., 2005; SOLIS-CALDERON et al., 2005; VONK NOORDEGRAAF et al., 2004).

Another disease with epidemiological characteristics is the bovine viral diarrhea virus (BVDV), a member of the family *Flaviviridae*, genus *Pestivirus* (BOLIN & RIDPATH, 1996). The main characteristic of this disease is the presence of animals persistently infected (PI) who become immunologically tolerant to BVDV and, therefore, a source of infection in the herd (DUBOVI, 1998).

The main risk factors associated with BVDV are: entry of a PI animal in the herd, healthy cows suffering from acute infection during pregnancy and the presence of transiently infected animals (GOYAL & RIDPATH, 2005). Other factors contributing to the spread of the disease in the herd are population density (SOLIS-CALDERON et al., 2005), the type of property, dairy or beef cattle (QUINCOZES et al., 2007) and the age of susceptible animals (MAINER-JAIME et al., 2001).

ELB is a contagious disease that affects cattle and has economic importance in the business. It is caused by a retrovirus that belongs to the family *Retroviridae*, genus *Deltaretrovirus* (LICURSI et al., 2003). Because it is a chronic disease, it usually affects animals older than two years (TRAININ et al., 2005). The infection may result in manifestations that can be academically separated into three distinct phases: the first, where the animal is asymptomatic (60 to 65%), but seroconversion can occur; the second phase, the animal also develops a persistent lymphocytosis (20-30%) characterized by an increase in the number of leukocytes and lymphocytes by three standard deviations above the normal readings in 90 days; and the third, the clinical phase, is characterized by the development of usually fatal malignant lymphoma (1 to 5%), (SAMARA et al., 1997; DUS SANTOS et al., 2007).

Therefore, the objective of this research was to verify how and if the occurrence of BoHV-1 changes

when the disease is associated with at least one immunosuppressive viral diseases, either BVD or EBL.

MATERIAL AND METHODS

The farms were selected by performing serological tests to determine the presence of antibodies against BoHV-1 in a representative sample of the herd. Six experimental farms located in Minas Gerais and São Paulo were used. Three of which were dairy farms, one beef cattle and one mixed herd, all of these had animals seropositive for BoHV-1. The sixth was a dairy farm that was used as control where no animal was seropositive. For this study, the herds selected were not vaccinated against BoHV-1 and BVDV-1.

Blood samples were drawn from all animals of the selected herds, by puncture of the median caudal or jugular vein, regardless of age. Subsequently, the blood samples were taken to the laboratory of viral reproduction, in the Departamento de Medicina Veterinária Preventiva, da UNESP – Jaboticabal, where serum was separated and stored in “eppendorf” flasks at -20°C until used, totaling 351 samples.

The virus neutralization test (VN) was the diagnosis method used to detect the presence of antibodies against BoHV-1, Nebraska strain, from the Universidade Estadual de Londrina - PR, and BVDV-1, Singer strain, from the Center of Rural Sciences, Universidade Federal de Santa Maria – RS, according to the protocol recommended by OIE (2007). The agar gel immunodiffusion (AGID) was the technique used to detect antibodies against ELB, following manufacturer's recommendations (TECPAR®, Paraná).

Chi-square (χ^2) test was used to analyze the data and to determine if there was correlation ($\alpha = 0.01$) of the diseases studied with cattle age; type of herd, dairy, beef or mixed, as well as possible association of BoHV-1 with BVDV-1 and EBL. The means were further compared by unfolding of the χ^2 test to check for significant differences regarding age and type of herd (ZAR, 1999).

RESULTS AND DISCUSSION

The results are presented in tables. A total of 351 blood samples were collected from herds of six different properties, small (P1, P2 and P3), medium size (P4 and P5) and the control farm (P6) (Table 1). From the properties studied, four were in São Paulo, P1, P3, P4 and P6, totaling 233 samples; 73 from herd P6, the control; and two were in Minas Gerais, totaling 118 samples. The results show the occurrence of three viral diseases, BoHV-1, BVD and EBL, which are widespread in much of the world.

The virus BoHV-1 was found in 54.7% (152/278) of the animals. According to studies by several authors, the prevalence of this virus in Brazil is between 28.9% and 82.7% (GALVÃO et al., 1963; MOLNÁR et al., 2001; ROCHA et al., 2001). In São Paulo the occurrence was 52.5% (84/160), and therefore very close to the 53.9% reported by Alfieri (1998). In Minas

Table 1 - Percent (%) of seropositive animals (R) to the enzootic bovine leukosis (EBL) by agar gel immunodiffusion analysis, along with geometric means (δ) of antibody titers obtained in virus neutralization tests for BoHV-1 and BVDV-1, according to herd type.

Farm	Herd type	N° of analyzed samples	BoHV-1			BVDV-1			ELB	
			R	%	δ	R	%	δ	R	%
P1	dairy	31	26	83.9	179	14	45.2	70	19	61.3
P2	dairy	42	34	80.9	86	30	71.3	63	9	21.4
P3	dairy	34	27	79.4	123	5	14,7	25	25	73,5
Total (dairy)		107	87	81,3	125	49	45,8	41	53	49,5
P4	Mixed	95	31	32,6	25	73	76,8	63	40	42,1
P5	beef	76	34	44,7	45	72	94,7	784	3	3,9
Sub-total		278	152	54,7	75	194	69,8	144	96	34,5
P6 (control)	Dairy	73	3	4,1	18	3	4,1	127	56	76,7
Total		351	242	44,2	68	197	56,1	94	152	43,3

Table 2. Percent (%) of animals seropositive for BoHV-1 and BVDV-1 by virus neutralization test and to ELB by agar gel immunodiffusion analysis, according to age and type of herd.

Type of herd	Disease					
	IBR		BVD		ELB	
	reagent/total	%	reagent/total	%	reagent/total	%
beef	34/76 ^A	44,7	72/76 ^A	94,2	3/76 ^A	3,9
dairy	87/107 ^B	81,3	49/107 ^B	45,8	53/107 ^B	49,5
mixed	31/95 ^A	32,6	73/95 ^C	76,8	40/95 ^B	42,1
age						
calves	13/35 ^a	37,1	14/35 ^a	40	0/35 ^a	0
Young animals	24/65 ^a	36,9	48/65 ^b	73,9	15/65 ^b	23,1
Adult animals	115/178 ^b	64,6	132/178 ^b	74,2	81/178 ^c	45,5

* different letters in the column indicate statistically significant difference ($\alpha = 0.01$)

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Gerais, the occurrence was 66.1% (68/118), and higher than the 58.2% found by Rocha et al. (2001) for the same state (Table 2).

As for BVDV-1, it was found in 69.8% (194/278) of the samples, close to the 66.32% reported by Quincozes et al. (2007) and 61.47% by Figueiredo et al. (1997), but different from the 47.7% found by Pituco & Del Fava (1998). In São Paulo, this disease occurred in 57.5% (92/160) of the samples, values

close to the 56.49% found by Samara et al. (2004); and in Minas Gerais, it was found in 86.4% (102/118) of the samples, a rate much higher than the 57.56% reported by Samara et al. (2004) for the same state (Table 2).

In this work, the general occurrence of ELB was 34.5% (96/278). For Minas Gerais, the occurrence was 10.2% (12/278), and for São Paulo 52.5% (84/160); in the latter, the results were similar to the 47.4% reported

by Megid et al. (2003). The occurrence of ELB in Brazil also varies widely, from at least 9.2% reported by Moraes et al. (1996) in Rio Grande do Sul, to a maximum of 70.9% in Minas Gerais (LEITE et al., 1984) (Table 2).

The statistical analysis regarding the BoHV-1 infection in different age groups showed that adult animals were the most affected. The calves and young animals displayed similar infection rates 37.1% (13/35) and 36.9% (24/65), respectively, and were not statistically different. Regarding the type of livestock, the dairy cattle were more seropositive, with a prevalence of 81.3% (87/107), followed by beef cattle with 44.7% (34/76) and the lowest rates were among the animals of the mixed herd with 32.6% (31/95). Different results were reported by Dias et al. (2008) who found higher prevalence in beef cattle, and the lowest in the mixed herd. The most probable causes for the results found in this study are the fact that dairy animals live longer and under a more intensive management. In addition, the management adopted for dairy animals allow the calves to be separated for longer periods from the adult animals.

According to Boelaert et al. (2005), two factors favor BoHV-1 infection in older animals: first, older animals are more likely to be exposed to the virus; the second factor may be related to the intensive management, which causes great stress on the animal. Stress promotes the release of the latent virus, resulting in a viremia, which can result in the virus being excreted to the environment, thus becoming free to infect other susceptible animals (LEMAIRE et al., 1994).

The results of the statistical analysis relating BVDV-1 with the type of livestock in the property were also significant. In the present study, the dairy, mixed and beef cattle were different among themselves, and the highest prevalence was found for beef cattle, 94.7% (72/76); followed by mixed, 76.8% (73/95) and the lowest occurrence was found in the dairy cattle 45.8% (49/107). These data differ from results obtained by Quincozes et al. (2007) who found the highest prevalence in the mixed herd, followed by beef cattle and the lowest in the dairy cattle. If the calves from zero to eight months are excluded due to possible interference of colostrum antibodies, age was not a determining factor in the prevalence of this disease, since 74.2% (132/178) of the cases were in adult animals while 73.9% (48/65) in young animals. These data corroborate the results reported by Quincozes et al. (2007), who also found that age was not a risk factor, but diverge from those reported by Mainar-Jaime et al. (2001) who found higher prevalence of BVDV-1 in adult animals.

The relationship between ELB with animal age and type of livestock in the property was significant. The highest prevalence, 49.5 (53/107) was observed for dairy cattle, followed by mixed herd with 42.1% (40/95) and only 3.9% (3/76) for beef cattle. Regarding age, all groups were significantly different from each other, and the older animals were the most affected with prevalence of 45.5% (81/178), followed by young

animals, 23.1% (15/65), and last the calves with 0% (0/35).

The results obtained in this study with respect to ELB are in agreement with the literature regarding older animals of the dairy herd, which are the most affected by this disease. According to Dus Santos et al. (2007), the main means of ELB transmission is by iatrogenic way, by management, such as vaccination without changing the needle, and palpation of several animals with the same glove, among others. Also because ELB is a chronic disease, it affects most often older animals (TRAININ et al., 2005).

Regarding the possible association of diseases, the highest rate was 25.5% (71/278) for BoHV-1 together with BVDV, 21.9% (61/278) for BVDV-1 only; followed by 14.4% (40/278) for the three viruses together; 11.9% (33/278) with no disease; 10.8% (30/278) for BoHV-1 with ELB; 7.9% (22/278) for BVDV-1 with ELB; 6.1% (17/278) for BoHV-1 only and 1.4% (4/278) for ELB. It should be reminded that these results were found for blood samples of animals that had not been vaccinated against BoHV-1 and BVDV-1.

The possible association of BoHV-1 and BVDV-1; and BoHV-1 with ELB was statistically significant. Despite finding a higher prevalence of animals affected with the BoHV-1 and BVDV-1 association compared to BoHV-1 and ELB, the statistical difference was significantly higher for the latter ($\alpha = 0.01$) compared to BoHV-1 associated with BVDV-1 ($\alpha = 0.05$). The results suggest that the farms where there are animals with one of the two diseases, either BVDV or ELB, the probability of BoHV-1 occurrence is higher compared to the farms where the viruses are not present. One explanation for this maybe the immunosuppression that affects animals by either BVDV or ELB.

The immunosuppression caused by BVDV can result in decreasing lymphocyte memory response to other pathogens (LAMONTAGNE et al., 1989); regardless whether it was a mild or acute infection, and thus increasing the pathogenicity of other microorganisms (HOLLAND et al., 1993). This situation provides a considerable increase in the incidence and severity of respiratory tract diseases, such as those caused by BoHV-1 (POTGIETER, 1997).

On the other hand, animals affected by ELB may have a smaller amount of IgM in the blood, in addition to producing nonspecific antibodies that indicates a possible deficiency in humoral immunity (TRAININ et al., 2005).

In BoHV-1 infection, the virus migrates to the lymph nodes where it remains latent. The animal then becomes a carrier and potential transmitter of this disease for the rest of its life (MUYLKENS et al., 2007). The latent virus can lead to subsequent and intermittent episodes of viral re-excretion by the animal, with or without clinical signs (FENNER et al., 1993). This re-excretion of virus may occur when the animal undergoes stress, causing a viremia (LEMAIRE et al., 1994). What determines the presence or absence of the re-excretion is the immune status of the animal. If the animal has enough antibodies to fight the virus

being released into the blood stream, the shedding of the virus will not occur; if however, the amount of antibodies is insufficient, the virus present in the blood is excreted into the environment, and can, consequently, infect other susceptible animals (LEMAIRE et al., 1994).

CONCLUSIONS

Based on the results of this study, it was concluded that the three viruses are widespread in the surveyed regions of Minas Gerais and São Paulo. The highest prevalence was found for BVDV-1 (69.78%) followed by BoHV-1 (54.68%) and the lowest ELB (34.53%); and the probability of infection by BoHV-1 was higher when the animals were also infected by either BVDV or ELB. Regarding age, the adult animals were the most affected by all diseases studied. Dairy cattle was affected the most by BoHV-1 and ELB; while beef cattle were affected by BVDV and less affected by ELB and the mixed herd farm showed average prevalence of BVD and ELB compared to the other herds. It was also the least affected by BoHV-1.

ACKNOWLEDGMENTS

Thanks are due to the laboratory technician Andréa Souza Ramos de Medeiros for her support in the laboratory and to CAPES and FAPESP (process nº 2006/55899-4) for the financial support.

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