DISTRIBUTION OF ANTIBODIES AGAINST *Neospora caninum*, BVDV AND BHV-1 AMONG COWS IN BRAZILIAN DAIRY HERDS WITH REPRODUCTIVE DISORDERS*

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ABSTRACT:- MINEO, T.W.P; ALENIUS, S.; NÄSLUND, K.; MONTASSIER, H.J; BJÖRKMAN, C. **Distribution of antibodies against *Neospora caninum*, BVDV and BHV-1 among cows in brazilian dairy herds with reproductive disorders.** [Distribuição de anticorpos contra *Neospora caninum*, BVD e BHV-1 entre vacas de rebanhos leiteiros brasileiros com desordens reprodutivas.] *Revista Brasileira de Parasitologia Veterinária*, v. 15, n. 4, p. 188-192, 2006. Department of Veterinary Pathology, Faculty of Agricultural and Veterinary Sciences, Universidade Estadual Paulista, Via de acesso Prof. Paulo Donato Castellane s/n, Jaboticabal, SP 14.884-900, Brazil. E-mail: tmineo@fcav.unesp.br

Neospora caninum, bovine viral diarrhea virus (BVDV) and bovine herpes virus 1 (BHV-1) are worldwide spread pathogens associated with reproductive problems in cattle. The present work aimed to observe the infection pattern of these three pathogens in two dairy herds with distinct reproductive managements from Triângulo Mineiro, Minas Gerais State, Brazil. The herds were not vaccinated against either *N. caninum*, BVDV or BHV-1. Blood samples were collected and analyzed for presence of specific antibodies, and *N. caninum* IgG avidity was measured in *N. caninum* positive samples. In herd 1, 34 out of 174 sampled cows (20%) had antibodies to *N. caninum* and the seropositivity of BVDV and BHV-1 were 62% and 86%, respectively. Of 69 sampled cows in herd 2. 7 (10%) had antibodies to *N. caninum*, and 49% and 39% were seropositive to BVDV and BHV-1, respectively. The IgG avidity profiles indicated that *N. caninum* had been present in both herds for some years and that herd 1 had an ongoing horizontal spread of the parasite. The results indicate that the studied reproductive pathogens were present in the herds and partly may have contributed to their reproductive problems.

KEY WORDS: Antibodies, *Neospora caninum*, BHV-1, BVDV, ELISA.

RESUMO

Neospora caninum, virus da diarréia viral bovina (BVDV) e herpes vírus bovino tipo 1 (BHV-1) são patógenos de distribuição mundial, associados a problemas reprodutivos em bovinos. O presente estudo objetivou a observação dos padrões de infecção destes três agentes no Triângulo Mineiro, MG, em dois rebanhos leiteiros que apresentavam diferentes tipos de manejo reprodutivo. Os rebanhos não adotavam vacinações para nenhum dos agentes. Amostras de soro foram colhidas e analisadas para a presença de anticorpos específicos, além da avaliação da avidez de anticorpos IgG em amostras positivas para *N. caninum*. O rebanho 1 apresentou 34/174 positivos para *N. caninum* (20%), 62% e 86% de positividade para BVDV e BHV-1, respectivamente. Das 69 amostras colhidas no rebanho 2, 7 (10%) possuíam anticorpos contra *N. caninum*, 49 % para BVDV e 39% para BHV-1. O perfil de avidez de IgG indicaram que *N. caninum* estava presente em ambos os rebanhos a alguns anos, porém o rebanho 1 demonstrou que havia um processo de infecção horizontal ainda ativa. Os resultados indicam que os patógenos estudados estavam presentes nos rebanhos analisados e contribuíram, em parte, para os problemas reprodutivos encontrados.

PALAVRAS-CHAVE: Anticorpos, *Neospora caninum*, BVDV, BHV-1, ELISA.

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INTRODUCTION

Neospora caninum is a protozoan parasite recognized as a major cause of bovine abortion (DUBEY, 2003). The parasite has a worldwide distribution and has also been reported in Brazil (GONDIM et al., 1999; CORBELLINI et al., 2002; LOCATELLI-DITTRICH et al., 2001). The dominant route of infection in cattle is transplacental transmission during pregnancy (DUBEY, 2003), and horizontal transmission by ingestion of oocysts shed in feces of dogs also occurs (McALLISTER et al., 1998). Recent studies show that also coyotes may infect cattle (GONDIM et al., 2004). Both cattle that are acutely and chronically infected with *N. caninum* may abort their foetuses or give birth to weak calves that die within the first month of life. However, the majority of infected cows give birth to clinically healthy but chronically infected calves (DUBEY, 2003). Presence of specific antibodies indicates that an individual is infected, and IgG avidity can be used to estimate how long the infection has been present and also give some indication about the relative importance of the different transmission routes in a herd (BJÖRKMAN et al., 1999).

Bovine viral diarrhea virus (BVDV) is a worldwide spread pathogen with great economical impact mainly related to the immunosuppressive effects of primary infections and to associated reproductive problems, such as repeat breeding, abortions, stillbirths, weak calves and congenital defects in calves (HOUE, 1999; FRAY et al., 2000). An important factor for the spread of the disease is the birth of a persistently infected (PI) calf, which remains infected throughout life, and very effectively disseminates the virus in its excretions and secretions. There are indications that 0.5 to 2 % of the cattle in endemic infected countries are PI and 60-85% of adult cattle are antibody positive (HOUE, 1999). In Brazil the antibody prevalence appears to be lower, varying from 33-56% in different areas (CANAL et al., 1998). In a recent study only 28% of the animals in 15 unvaccinated herds were antibody positive to BVDV (MELO et al., 2004).

Bovine herpes virus 1 (BHV-1), also known as infectious bovine rhinotracheitis (IBR) virus, is causing respiratory and reproductive infections in cattle all over the world. Animals once infected by the virus are infected throughout life and BHV-1 remains in a latent state in the sciatic and trigeminal ganglia and tonsils (ACKERMAN et al., 1982). The virus can be reactivated by stress or administration of immunosuppressive hormones (SNOWDON, 1965). Abortions due to BHV-1 infection have been reported to occur throughout the gestation, being more frequently observed in its final stage. In Brazil, prevalence studies indicate a wide range of positivity to BHV-1, varying from 29 to 86% (D'ARCE et al., 2002, MELO et al., 2004).

The present work aimed to describe the serological status and observe possible correlations of *N. caninum*, BVDV and BHV-1 among cows of two dairy herds from Triângulo Mineiro in the State of Minas Gerais State, Brazil.

MATERIAL AND METHODS

Animals and sampling

The study was performed in two dairy herds in the Triângulo Mineiro region in the State of Minas Gerais, Brazil during the year 2000. Reproductive disorders consisted of repeat breeding, abortions, stillbirths, and birth of weak calves. The animals were maintained with intensive grazing and given a daily mineral supplementation rich in phosphorus. Farms were free of *Brucella abortus*, and still maintained vaccination programs, following Brazilian Agricultural Ministry's exigency. However, no other vaccines were used in the herds. The herds´ clinical records for the month prior to blood sampling were collected and all cows that had suffered from any reproductive disorder during this period were blood sampled. So were also the cows that had been in close contact with them (i.e. belonged to the same dairy lot, shared pasture, or were milked at the same time). Included were only cows that had been confirmed pregnant at least once; they were all of Holstein-Friesian breed.

Herd 1 had a total of 220 animals at time of sampling, including cows, heifers, bulls and calves, of which 174 cows were blood sampled. The cows were milked twice daily and the farm utilized manly artificial insemination and embryo transfer to breed its cows. During the month prior to sampling, 9 cows in the herd had shown reproduction related clinical signs; 1 aborted, 2 delivered stillborn calves and 6 cows gave birth to weak calves which died within a month after birth. In Herd 2, 69 cows out of a total of 120 animals were sampled. The cows were milked three times a day and were bred only by Holstein-Friesian bulls three months after the end of the previous gestational period. Three cows in this herd had aborted the month before sampling according to the records. Artificial inseminations were used occasionally in this herd, although in a much lower rate than Herd 1.

Blood was collected from the jugular vein and stored on ice until centrifuged at 500 x *g* for 5 minutes. The sera were stored at -20°C until used for the serological assays.

The investigations were performed according to the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation and the 2000 Report of the AVMA Panel on Euthanasia (AMERICAN VETERINARY MEDICAL ASSOCIATION, 2001).

Serology

Demonstration of antibodies to *N. caninum* was done essentially according to BJÖRKMAN and colleagues (1997) using proteins incorporated into immunostimulation complexes (iscoms) as antigen. Briefly, 96-weel plates were incubated with antigen overnight, at 4°C. Blockage of unspecific binding sites were realized with 10% of normal equine serum in PBS with 0,05% Tween 20 (Sigma Co., St. Louis, MO, USA). Serum samples were incubated at a 1:100 dilution and a monoclonal antibody to bovine IgG1 labeled with peroxidase (SVANOVA Biotech AB, Uppsala, Sweden) were used as conjugate. The reaction was revealed by TMB and H_2O_2 solution and stopped

with 10% sulfuric acid. Reaction was read at 450 nm in a plate reader (Labsystems AB, Stockholm, Sweden). All OD values were correlated to a positive control serum with mean OD of 1.0. Serum samples with corrected OD ≥ 0.20 were considered positive.

Neospora caninum IgG avidity ELISA. Sera that had OD values over 0.40 by the *N. caninum* ELISA were examined further by an IgG avidity test (BJÖRKMAN et al.,1999) to differentiate between recent and chronic infections. The avidity results in each herd were clustered into classes according to Björkman et al. (2003).

Analysis for presence of antibodies to BVDV was performed by BVDV-Ab SVANOVIR ELISA kit (SVANOVA Biotech AB, Uppsala, Sweden) following the manufacturer's specifications. Briefly, serum samples were incubated at a 1:100 dilution and a monoclonal antibody to bovine IgG1 labeled with peroxidase was used as conjugate. The reaction was revealed by TMB and H_2O_2 solution and stopped with 10% sulfuric acid. Reaction was read at 450 nm in a plate reader (Labsystems AB, Stockholm, Sweden). Sera with optical density values (OD) ≥ 0.20 were deemed positive.

The ELISA kit IBR-Ab SVANOVIR (SVANOVA Biotech AB, Uppsala, Sweden) was used to detect antibodies against BHV-1. The test was carried out following the manufacturer's specifications. Serum samples were incubated at a 1:25 dilution and a monoclonal antibody to bovine IgG1 labeled with peroxidase was used as conjugate. The reaction was revealed by TMB and H_2O_2 solution and stopped with 10% sulfuric acid. Reaction was read at 450 nm in a plate reader (Labsystems AB, Stockholm, Sweden). Samples were considered positive when OD were ≥ 0.20.

Statistical analysis

Seroprevalence among age groups and associations between agents in within the two different herds were analyzed by non-parametrical Fisher exact test using SAS V8 statistical software for Windows (SAS Institute, NC, USA).

RESULTS

The 174 sampled cows from Herd 1 had a mean age of 9.5 years. Thirty four of them (20%) had antibodies against *N. caninum* (Table 1). The younger cows, i.e. age group 3-6 years, had a higher level of seropositivity when compared with the older cows ($p<0.05$). There were both low and high avidity animals in the herd (Figure 1a). The seroprevalence for BHV-1 was 86% (150/174), and 108 (62%) of the cows had antibodies to BVDV (Table 1). There was no difference between the age groups regarding seroprevalence for either BHV-1 or BVDV in the herd. The cow that aborted was seropositive to *N. caninum*, with OD=0.98 and IgG avidity 98. From the remaining animals with reproductive disorders, seven of the 9 cows were positive to BVDV and all were positive for BHV-1.

In Herd 2 mean age of the 69 sampled cows was 6.8 years. Seven cows in the herd (10%) had antibodies against *N. caninum* and all of them had high IgG avidity values (Figure

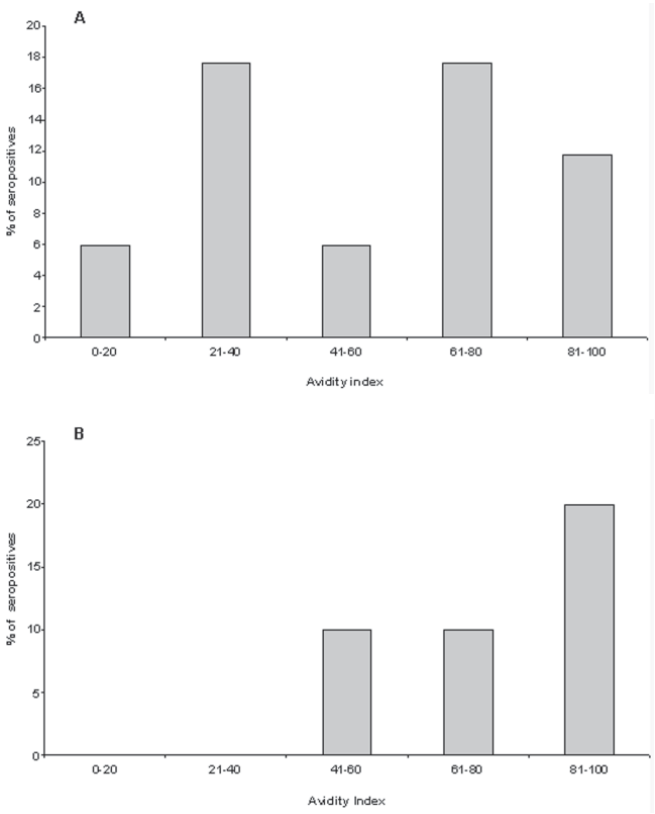

Figure 1. *Neospora caninum* IgG avidity in cows from two cattle herds with clinical history of reproductive disorders; Herd 1 (**A**) and Herd 2 (**B**).

Table 1. Seropositivity to *Neospora caninum*, Bovine Viral Diarrhea Virus (BVDV) and Bovine Herpes Virus 1 (BHV-1) in two dairy cattle herds with history of reproductive disorders, by age.

Age (years)	Herd 1			Herd 2		
	N. caninum % (pos/total)	BVDV % (pos/total)	BHV-1 % (pos/total)	*N. caninum* % (pos/total)	BVDV % (pos/total)	BHV-1 % (pos/total)
3-6	60 (6/10)	70 (7/10)	90 (9/10)	7 (2/29)	38 (11/29)	24 (6/25)
7-10	19 (23/123)	64 (79/123)	85 (104/123)	14 (5/36)	56 (20/36)	52 (14/27)
≥11	12 (5/41)	54 (22/41)	90 (37/41)	0 (0/4)	75 (3/4)	50 (2/4)
Total	20 (34/174)	62 (108/174)	86 (150/174)	10 (7/69)	49 (34/69)	39 (22/56)

3-6 years: cows considered in initial reproductive phase.
7-10 years: cows in mid-reproductive age.
>11 years: cows with declining reproductive activity.

1b). BVDV was the most prevalent infectious agent in this herd and 34 of the sampled cows (49%) had antibodies to the virus. Of the 56 samples that were tested for BHV-1, 22 (39%) were positive (Table 1). Thirteen samples were not tested for antibodies to BHV-1 due to a small quantity of serum available. One of the cows that aborted in this herd was positive to *N. caninum* and all 3 were negative to BVDV. One out of two other aborting animals was positive to BHV-1. There were no differences between the age groups regarding seroprevalence for any of the three investigated agents in the herd. Additionally, no statistical associations were found between presence of antibodies to the different agents tested in either Herd 1 or Herd 2.

DISCUSSION

The results in this study show that both herds were infected with *N. caninum* and BHV-1, and have been exposed to BVDV. The prevalences of *N. caninum* were within the same range as previously been reported from various regions of Brazil (GONDIM et al., 1999; LOCATELLI-DITTRICH et al., 2001; CORBELLINI et al., 2002). The mean seroprevalence (17%) was similar to the 12% recently found in other unvaccinated herds in the region. It was, however, considerably lower than the 46% in 3 herds that used BVDV and BHV-1 vaccines (MELO et al., 2004). The reason for this higher apparent *N. caninum* infection rate in the vaccinating herds is not known. They might already before starting the vaccination program have had a higher infection rate than the non-vaccinating herds.

In order to better understand the epidemiology of *N. caninum* in the herds in this study, the positive samples were analysed for *N. caninum* IgG avidity. The avidity reflects the duration of infection and herd avidity profiles, visualizing the distribution of the seropositive cows among IgG avidity classes, can be used to estimate the relative importance of vertical and horizontal transmission (BJÖRKMAN et al., 1999, 2003). Herd 1 had both low- and high-avidity cows. The cluster of seropositive cows with avidity values between 20 and 40, and the presence individuals with very low avidities, suggest that there was an ongoing horizontal spread of the parasite. However, the fact that 30% of the infected cows had avidity values >60 indicates that the infection was not recently introduced, but had been present in the herd for some years. It is worth noting that the only cow in Herd 1 that had aborted during the month prior to the blood sampling was *N. caninum* positive with a high antibody and avidity levels. In Herd 2, on the other hand, all positive cows had high avidity values indicating that the predominant route of infection was congenital transmission (BJÖRKMAN et al., 2003).

BVDV is one of the most important virus infections in cattle world-wide, and is causing huge economic losses in countries endemically infected with the virus (KOVACS et al., 2003). The 58% prevalence of antibodies against BVDV in this study was similar to what has been found in previous investigations in Brazil (28 to 73%: CANAL et al., 1998; MELO et al., 2004). However, it was rather low considering the high mean age of the tested animals, and suggests that there were no persistently infected animals in the herds, since it is typically observed in herds with PI animals between 90 to 100% of the older animals are seropositive (HOUE, 1999). In an earlier study performed in the State of Minas Gerais by Melo et al. (2004), only 1 out of 15 herds that were not vaccinating against BVDV had prevalence > 80%, and no antibody positive animals were found in 2 herds. Taken together, the results of these 2 studies suggest that a majority of the unvaccinated herds in this region will eventually become spontaneously free from BVDV due to self-clearance. That is, if proper biosecurity measures are taken and no PI animals or pregnant dams carrying PI fetuses are introduced to the herds (LINDBERG;ALENIUS, 1999). During infections with BVDV and BHV-1, a majority of the animals in a herd are commonly infected before the age of two years and develop a lifelong antibody response (BIUK-RUDAN et al., 1999).

Previous seroepidemiological studies have shown that between 10 to 50% of the Brazilian cattle population have antibodies against BHV-1 (D'ARCE et al., 2002). In the present investigation, Herd 1 had a higher seroprevalence (86%) than Herd 2 (39%). The reason for the higher prevalence in Herd 1 might be that it had been in contact with, or introduced, an animal with active virus infection. Alternatively, healthy carriers of BHV-1 can have been exposed to stressing conditions, resulting in reactivation and spread of the infection to susceptible animals in the herd (AFSHAR;EAGLESOME, 1990).

The principles for eradication of BVDV from cattle herds without vaccination used in Scandinavia have been very successful and they are well on their way towards eradication of BVDV (LINDBERG;ALENIUS, 1999) and control measures to reduce the impact of *N. caninum* infections are discussed (FRÖSSLING et al., 2005). It is probable that an increased awareness of biosecurity and hygiene measures among the farmers in this area of Brazil could be very effective in reducing the overall impact of BVDV and other reproductive pathogens in the cattle herds. Introduction of new animals without appropriate testing for BVDV could result in outbreaks of diseases that could seriously compromise the reproductive health of the animals (PRITCHARD et al., 1989; MURRAY, 1990). Farmers must be made aware of the improvements in animal health that can be gained from disease clearance and that they themselves are responsible for the herd's biosecurity.

This investigation revealed that the studied reproductive pathogens were present in the herds and partly may have contributed to their long lasting reproductive problems. Although no positive association was found between the pathogens, it is still necessary to verify if agent interactions may be responsible for bovine gestation miscarrying. Also, further studies are needed to develop cost effective ways to control these infections health in cattle herds in Brazil and other South American countries.

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