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Monitoring bovine viral diarrhea virus (BVDV) infection status in dairy herds[1]

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ABSTRACT.- Diéguez F.J., Yus E., Sanjuán M.L., Vilar M.J. & Arnaiz I. 2008. **Monitoring bovine viral diarrhea virus (BVDV) infection status in dairy herds.** *Pesquisa Veterinária Brasileira 28(12):588-592.* Unidad de Epidemiología y Sanidad Animal, Instituto de Investigación y Análisis Alimentarios, Facultad de Veterinaria s/n, Lugo 27002, Spain. E-mail**:** franciscojavier.dieguez@usc.es

This study was designed to assess the relationship between antibodies against bovine viral diarrhea virus (BVDV) determined in the bulk tank milk (BTM) and the within-herd seroprevalence. We also assessed the efficiency of measuring antibody levels in BTM samples to monitor BVDV infection status in a herd. In the 81 farms included in the study, BTM samples were obtained and blood samples withdrawn from all cattle older than one year. The infection status was then determined in serum and milk using a commercial blocking ELISA based on the detection of anti-p80 antibodies. Apart from these baseline serum and milk samples, another BTM sample was collected from each herd 9 months later, and a third BTM sample obtained 9 months after this. In these second and third milk samples, anti-BVDV antibodies were determined using the same ELISA kit. Statistical tests revealed good agreement between herd seroprevalences (% seropositive animals in the herd) and the antibody levels detected in the BTM samples. During the 18 months of follow-up, the farms with persistently infected cattle at the study outset (14.8% of the herds) showed a significant decrease in BTM antibody titers after virus clearance. Conversely, a significant increase in BTM antibody levels was observed in the herds infected with BVDV during the follow-up period. Our findings indicate that monitoring antibody levels in the BTM is a useful method of identifying changes in the BVDV infection status of a herd.

INDEX TERMS: bovine viral diarrhea vírus, BVDV, antibodies, diagnostic, milk.

RESUMO.- [**Monitoramento do estado de infecção pelo vírus da diarrhéia viral bovina (BVDV) em rebanhos bovinos leiteiros.**] Os objetivos do presente estudo foram avaliar a relação entre os níveis de anticorpos frente ao vírus da diarréia viral bovina (BVDV) no tanque de leite e a prevalência de animais seropositivos em cada rebanho; e também avaliar a eficiência da medição dos níveis de anticorpos no tanque de leite como método de monitoramento do *status* de infecção frente ao BVDV. Nos rebanhos estudados, obtiveram-se amostras de soro de todos os animais com idade superior a um ano, assim como uma amostra de tanque coletivo de leite. As amostras de soro e leite foram analisados por um teste ELISA de bloqueio baseado na detecção de anticorpos anti-p80. Posteriormente coletaram-se mais duas amostras do tanque de leite em cada exploração com intervalos de nove meses entre as coletas. Estas amostras foram analisadas com o mesmo ELISA. A análise estatística mostrou uma boa relação entre a soroprevalência dos rebanhos e a percentagem de inibição na amostra de tanque de leite. No decorrer do procedimento, aquelas explorações que possuíam animais PI no início do estudo (que representavam 14.8% dos rebanhos estudados) mostraram um decréscimo estatísticamente significativo dos níveis de anti-

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corpos após a eliminação dos animais persistentemente infectados. Ao contrário, as explorações que sofreram a introdução da infecção durante o estudo mostram um incremento significativo nos níveis de anticorpos no leite. Nossas conclusões indicam que a avaliação de níveis de anticorpos no tanque de leite é um método útil de identificar mudanças do estado sorológico da infecção BVDV de rebanhos leiteiros.

TERMOS DE INDEXAÇÃO: vírus da diarréia viral bovina, BVDV, anticorpos, diagnóstico, leite.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is a widespread pestivirus infection affecting mainly cattle but also other ruminants (Carlsson 1991). The disease causes considerable economic losses in the dairy industry, mainly attributable to reduced milk production, reduced reproductive performance, delayed growth, increased susceptibility to other diseases, early culling and increased mortality among young stock (Houe 2003). Infections in susceptible adult cattle are often subclinical, although this depends on the causative virus strain. Normally, only transient mild fever and leukopenia can be observed in closely examined animals. Two to three weeks after infection, neutralizing antibodies are produced (Howard 1990). However, when the infection occurs in a susceptible pregnant cow, the fetus may be infected, and the consequences of this depend on the precise moment infection takes place. Thus, it could lead to embryonic death, abortion, congenital defects or stillbirth, or to the birth of persistently infected (PI) calves. The latter occurs when the fetus is infected in the first trimester of pregnancy (mostly from 30 to 90 days of gestation) due to the development of specific immunotolerance against BVDV (Moennig & Liess 1995). PI animals shed large amounts of virus during their lifetime, to the extent that they are the main infection source of BVDV (Meyling et al. 1990). Normally, in the presence of a PI animal, seroconversion will occur in all animals with which it comes into contact.

To estimate the prevalence of BVDV antibody carriers in a herd, ELISA can be used to detect antibodies both in individual samples of serum or milk (Beaudeau et al. 2001). The use of this technique to measure antibody titers in the bulk tank milk (BTM) has also been recognized as a valuable tool for this purpose (Niskanen 1993).

In any BVDV control program, farms with active infections in which the presence of a PI animal is highly probable should be identified to eliminate these animals (Greiser-Wilke et al. 2003). To monitor the infection status of farms, we speculated that a cheap, rapid and effective method could be to determine the levels of antibodies against BVDV in BTM. Antibody patterns detected in serial BTM samples could be used to identify events, such as a new active BVDV infection, that could compromise the profitability of a herd. Suitable sampling intervals will depend on the infection risk of each farm.

The aim of the present study was to evaluate the efficiency of serial BTM ELISA antibody detection as a method of monitoring the BVDV status of a dairy herd and to examine the relationship between within-herd seroprevalence and BTM levels of antibodies against the virus.

MATERIALS AND METHODS

Dairy herds

This study was performed over an 18-month period (2004-2005) in Galicia (NW Spain), the country's largest dairy region. In the year 2004, milk production in Galicia accounted for 35% of all the milk produced in Spain. Cattle disease control programs in Galicia are voluntary and conducted through an organization established to improve livestock health (Agrupaciones de Defensa Sanitaria Ganadera, ADSG), which has been working since 2004. The BVDV program, the first of its kind in Spain, is mainly based on detecting and eliminating PI animals along with the strict control of purchased cattle. When permitted, only inactivated virus vaccines are used.

For this study, we selected 81 dairy farms by simple random sampling of farms that had started a BVDV control program. These 81 farms comprised 4512 Holstein-Friesian cows older than one year representing a mean of 55.7 (SD=38.3) animals/farm.

Study design and antibody detection in serum and bulk tank milk samples

In each farm, blood was withdrawn from all cattle older than one year and antibodies against the p80 antigen (BVD/MD p80, Pourquier laboratories) determined in serum samples using a commercial blocking ELISA according to the manufacturer's instructions. In animals vaccinated with inactivated vaccines, the antibodies mainly react with structural proteins rather than the p125 or p80 antigens (Bolin & Ridpath 1990). This allowed for differentiation between wild type BVDV and the vaccine virus since live vaccines are not used in the herds examined here. At the same time, BTM samples were collected from each herd and tested for anti-BVDV antibodies using the same ELISA kit. When tested blood samples indicated a possible PI (i.e., when a positive result was obtained in a young heifer), this was confirmed by antigen capture ELISA (Antigen serum plus BVD test kit, IDEXX laboratories) based on the detection of the E^{rns} viral protein. All PI cows identified were immediately culled and all calves born to the herd during the following year were tested for the virus as described above and newborns scoring positive for BVDV were culled, while those yielded two negative antigen ELISA results 2-3 weeks apart were returned to the herd. Nine months after obtaining the baseline serum and milk samples, a second BTM sample was obtained, and a third milk sample obtained 9 months after this. These BTM samples were analyzed using the same ELISA to detect anti-BVDV antibodies. The results of the BTM tests were expressed as percentage inhibition values calculated according to the optical densities (OD) measured at 450

nm of the samples and the negative control provided in the kit as follows:

Percentage = (OD450 sample/mean OD450 negative control) 100
inhibition

The first set of samples was used to compare herd seroprevalences (defined as the % seropositive animals older than one year in each farm) with the percentage inhibition results obtained in the BTM. These percentage inhibitions were interpreted according to the prevalence thresholds proposed by Eiras et al. (2005) for the same geographical region as follows: a percentage inhibition greater than 88% indicates a herd prevalence of 0% to 5%; a percentage inhibition 58% to 88% a herd prevalence of 5%-25%; a percentage inhibition 22%-57% a herd prevalence of 25%-65%; and a percentage inhibition less than 22% a herd prevalence higher than 65%.

Statistical analysis

All data were processed using SPSS 12.0 software. The k index was used to assess agreement between herd seroprevalences and percentage inhibition data obtained for BTM samples using a categorical approach. The ñ was reported as a measure of linear association between herd seroprevalence and percentage inhibition recorded in milk using a quantitative approach. The non-parametric Jonckheere-Terpstra (J-T) or ANOVA tests were conducted to examine changes in the percentage inhibition data obtained in the serial BTM samples.

RESULTS

Good agreement was observed between the herd seroprevalences recorded and the percentage inhibition data obtained in the milk samples (Fig.1). The categorical method yielded linear and quadratic k values of 0.62 (CI 95% = 0.48-075, P<0.00001) and 0.73 (CI 95% = 0.58-0.87, P<0.00001) respectively. The ñ value indicated by the quantitative approach was 0.72 (CI 95% = 0.58-0.82, P<0.00001).

Based on the serological profiles of the herds, 12 of the 81 dairy farms were suspected of harboring an active infection at the study outset. In these farms, at least one PI animal was detected using the antigen ELISA kit although the number of PI animals identified per farm was 1 to 5. The age of these PI animals ranged from 1 month to 5 years. Accordingly, at the start of the BVDV control programs established on each farm, the prevalence of herds with PI animals was estimated at 14.8% (12/81). The remaining 69 farms were classified as being free of active BVDV infection according to the serological tests. Notwithstanding, during the first 9 months of follow-up (between obtaining the first and second BTM samples), BVDV was somehow introduced in 4 of these farms. The results of the second BTM samples made us suspect these new active infections, which were subsequently confirmed by obtaining serum samples from heifers 9-15 months old for antibody ELISA testing and confirming PI animals by antigen ELISA.

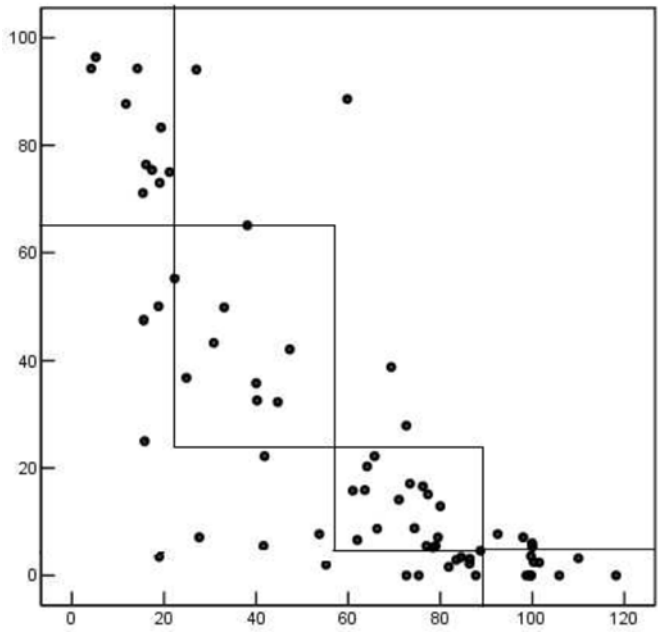

Fig.1. Agreement between herd seroprevalences and percentage inhibition values recorded in BTM. Lines indicate the cut-off values proposed by Eiras et al. (2005).

In 3 of the farms in which the virus appeared in the first follow-up session (second BTM milk sample), it was possible to demonstrate that the infection source was the purchase of untested cattle (these were later identified as PI cows or cows carrying PI fetuses). The fourth farm had not acquired any replacement animals since 1995 and the animals had not come into contact with cattle from other farms. However, the timing of viral infection did coincide with the purchase of four sheep; however, although we were unable to confirm that this was the infection source since the virus could not be isolated in these animals.

Table 1. Percentage inhibitions (as determined by a blocking ELISA) recorded in BTM samples from dairy herds in Galicia that were actively infected with BVDV at the study outset but were able to clear the virus

Farm designation	% Inhibition 1st BTM sample[a]	% Inhibition 2nd BTM sample	% Inhibition 3rd BTM sample
1	-	13.7	39.2
2	-	52.8	69.7
3	38.1	42.7	75.3
4	5.2	12.2	29.3
5	4.2	5.2	16.7
6	15.4	37.7	37.0
7	11.7	11.5	44.1
8	15.6	15.1	39.8
9	16.1	17.0	19.0
10	14.2	16.3	26.5
11	-	13.7	39.2
12	-	52.8	69.7
Mean (CI 95%)[b]	15.1 (12.0-18.2)	20.5 (17.7-23.3)	35.9 (32.3-39.5)

[a] Nine month intervals between samples.
[b] P=0.001, significant increase in percentage inhibition after virus clearance.

Table 2. Percentage inhibitions (as determined by a blocking ELISA) recorded in BTM samples from dairy herds in Galicia in which BVDV was detected during follow-up

Farm designation	% Inhibition 1st BTM sample[a]	% Inhibition 2nd BTM sample	% Inhibition 3rd BTM sample
13	69.3	55.1	18.2
14	86.4	40.4	23.3
15	42.3	8.6	14.9
16	98.7	11.7	13.7
Mean	74.2	28.9	17.5
(CI 95%)[b]	(54.7-93.6)	(10.9-46.8)	(14.1-20.9)

[a] Nine month intervals between samples.
[b] P=0.028, significant decrease in percentage inhibition after herd entry of the virus.

Table 3. Mean percentage inhibitions (as determined by a blocking ELISA) recorded in BTM obtained from dairy herds in Galicia in which active BVDV was not detected during the study

BTM sample[a]	N	Percentage inhibition (mean)[b]	CI (95%)	
			Lower limit	Upper limit
1st	53	69.3	61.9	76.6
2nd	65	65.8	59.6	72.1
3rd	65	80.8	74.8	86.8

[a] Nine month intervals between samples. P=0.001, significant increase in percentage inhibitions between the first/second and third samples.

In the 12 dairy farms with active BVDV infections at the start of the study, any PI animals detected were eliminated from the herd, and after testing calves born to the remaining animals in the following year, no new PI animals were detected. In these farms from which the virus was cleared, we observed that percentage inhibition values obtained in the BTM increased significantly over the 18-month follow-up period (Table 1). In the 4 dairy farms in which the virus appeared during follow-up, the first tank milk samples rendered high percentage inhibitions while these values dropped significantly after entry of the virus (Table 2). The remaining 65 farms, in which no active virus was observed during the entire study, always displayed percentage inhibitions in the bulk tank higher than 60. The differences between the first and second BTM samples were not significant, but significant increases were noted between the first/second and third samples (Table 3).

DISCUSSION

Using an indirect ELISA, Niskanen (1993) reported a direct relationship between the prevalence in herds of cows that were positive for the anti-BVDV antibody and antibody titers recorded in BTM. Our results support this idea that antibodies in BTM can be a valuable tool for estimating the prevalence of this viral disease in a herd. However, in both these studies, strong disagreement between herd seroprevalence and milk percentage inhibition values was observed in a small proportion of cases. A few animals with high antibody serotiters could lead to high antibodies levels in the bulk milk, causing overestimation of the number of seropositive animals (Frediksen et al. 1998). On the contrary, an underestimated herd seroprevalence could be the result of viruses from PI animals neutralizing antibodies in the BTM (Niskanen 1995). Over- or underestimates can also sporadically arise because of cows being milked at the time the BTM is obtained, such that the sample is not representative of the herd BVDV status. In other cases, disagreement can be attributed to inadequate preservation or storage of the milk samples before they are processed.

Thus, despite the generally good correlation between sera and BTM results, these confounding factors and the fact that BTM samples preclude knowing the age of the BVDV-positive animals identified, determine that, by testing only one BTM sample, it cannot be reliably determined if a herd has an active infection. Hence, one BTM test does not serve to distinguish actively infected herds from herds early infected, but cleared of the virus (Valle et al. 2001). Moreover, if the infection is recent, levels of antibodies in BTM could still be low. It is also possible that PI animals transiently shed low amounts of virus and therefore slowly transmit the infection (Thurmond 2005). In contrast, when the results of two or more consecutive BTM samples are available, these will alert us of any change in the BVDV status of a herd and help decide if further investigations are needed.

In the dairy farms harboring active infections that showed high BTM antibody levels, once the virus had been cleared, antibodies gradually fell. Notwithstanding, it is known that after virus elimination, infected cows remain seropositive for years. Probably in the absence of a virus source for continuous reinfections, the herd stops constantly producing antibodies. Besides, there will be an ever-increasing number of lactating seronegative cows contributing to the bulk milk. Our data indicate a greater decrease in milk antibody levels between the second and the third sampling times than between the first and second, since all PI animals were identified and eliminated during the first 9 months of follow-up.

In herds in which serological profiles indicate no active infection, any new infection can easily be detected according to antibody level changes produced in the BTM. Accordingly, the present farms in which the virus was found to appear during the follow-up period, showed a decrease in percentage inhibition values that was overall significant. This increase in antibody levels points to a source of infection in the herd (the most effective being one or more PI animal(s)). In one of the 4 farms in which the virus was introduced, the increase in antibodies was not as significant as in the rest, because the BTM sampled corresponded to a recent infection. It follows that when monitoring a farm for BVDV status, even a modest drop in the percentage inhibition value will warrant further investigation. On this farm, BTM sampling was capable of detecting early infection. In the 4 farms undergoing infection during follow-up, the result of the third BTM

sample depended on whether or not the virus had been cleared.

On the whole, the 65 farms in which active infections were not observed during the study, showed a trend toward improved percentage inhibitions during the follow-up period. This probably reflects the fact that the farmers adopting BVDV control programs took adequate measures to ensure seronegative herds. However, some of these farms (7 or 10.8% of the 65 farms) showed a slightly reduced percentage inhibition in one milk sample with respect to the previous sample. This situation could be erroneously interpreted as a recently acquired infection. Such discrepancies could be explained by varying contributions of individual cows to the BTM, different milk yields of antibody negative and positive cows or could even reflect the incorporation of new seropositive animals (Rikula et al. 2005) in farms that were less committed to the control program. These factors should therefore be considered when interpreting BTM Ab-ELISA results, especially if the farm in question is small.

In conclusion, our findings indicate that, as part of an active BVDV control program, regular testing of BTM samples for antibodies against BVDV is a reliable way to monitor the BVDV status of dairy herds and alert the farmer of the need for additional testing.

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