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PREVALENCE OF ANTIBODIES TO IBR AND BVD VIRUSES IN DAIRY COWS WITH REPRODUCTIVE DISORDERS

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ABSTRACT

We determined the prevalence of antibodies to infectious bovine rhinotracheitis virus (IBRV) and bovine viral diarrhea virus (BVDV) in sera of dairy cows on 4 different farms in the Republic of Croatia. A high percentage (60.8%) of cows had various reproductive disorders. The results showed that seroprevalence of infectious bovine rhinotracheitis (IBR) was 85.8% and that of bovine viral diarrhea (BVD) was 79.2% in tested cows. Antibodies to both viruses were found in 80.8% of cows with reproductive disorders but in only 46.8% of cows without reproductive disorders. This difference was statistically significant ($P<0.01$), and indicated a connection between reproductive disorders and simultaneous infections with IBR and BVD viruses in dairy cows.

Key words: infectious bovine rhinotracheitis, bovine viral diarrhea, dairy cows, reproductive disorders

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) are diseases with a worldwide distribution in domestic and wild ruminants and result in severe economic losses to the cattle industry.

Infectious bovine rhinotracheitis is caused by bovine herpesvirus 1 (BHV-1), which may also cause conjunctivitis, meningoencephalitis, infectious pustular vulvovaginitis and balanoposthitis, abortions and systemic infections (11). Although there is no definite association between sub-type and the clinical entity, BHV-1 sub-types 1 and 2a are the main causes of the respiratory form of the disease and, frequently, of abortion. However, sub-type 2b is responsible for infectious pustular vulvovaginitis and infectious pustular balanoposthitis (23). Abortions mostly occur during the third trimester of pregnancy. Infertility and shortened estrous cycles have been observed in nonpregnant cows inseminated at estrus with semen containing IBR virus. The IBR

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virus causes limited necrotising endometritis and necrotising oophoritis. Usually, both ovaries are affected by IBRV infection, although the most severe lesions begin on the corpus luteum (cl; 17, 18, 19, 22).

The severity of bovine viral diarrhea in cattle ranges from transient acute infections, which may be unapparent or mild, to a mucosal disease that is inevitably fatal. Venereal infection with BVD virus is shown to be an important factor in the transfer of virus to the fetus. Congenital infections can cause abortion, mummification, stillbirth, malformation and the development of persistently viraemic calves (7). Abortions usually occur between 50 and 100 d of gestation. Acute and persistently infected bulls may play a great role in the spread of BVD due to virus excretion by semen. Infection at the time of breeding through using BVDV-infected semen causes reduced conception rate, which is most probably due to reduced fertilization. Moreover, BVDV induces inflammation of ovaries and pustular lesions on genital organs, similar to BHV-1 (3,4,6,12).

The mucosal disease arises from initial fetal infection with a non-cytopathogenic virus and the subsequent production of persistently viraemic calves. These calves may later develop mucosal disease as a result of superinfection with a "homologous" cytopathogenic virus (7).

Concurrent infections of BVD virus and other respiratory or enteric pathogens may produce a more severe disease than either pathogen alone because of the immunosuppressive effect of BVD virus (1,20).

With regard to the foregoing, the aim of this study was to determine seroprevalence to IBR and BVD viruses in the group of dairy cows with reproductive disorders and the group with no reproductive disorders. That is to say, in combined infections of IBR and BVD viruses the immunosuppressive effect of BVD virus may be a triggering factor for activation of IBR virus in latently infected cattle.

MATERIALS AND METHODS

The sample for this study comprised 120 Holstein-Friesian cows, aged 2 to 9 yr, kept at 4 different dairy farms in the Republic of Croatia. Average annual milk production on these farms was 6,000 L per cow.

These farms were chosen over others because of frequent reproductive problems that had occured over the few last years. We have tested for one group of cows with reproductive disorders and a second group without reproductive disorders from each farm. These groups were made by cluster random sampling, and cows within groups were chosen by systematic random sampling.

Blood samples were collected from all dairy cows by puncture of the jugular vein into tubes without an anticoagulant. All blood samples were centrifuged in the laboratory at 1,200 rpm for 10 min to remove the sera, which were then heat-inactivated at 56 °C for 30 min prior to testing.

Sera were examined for IBR and BVD viruses by serum-neutralization test. Antibodies were determined by the micro-method, using Linbro IS-FB-96 plates. Four serial two-fold dilutions of each serum were incubated in 50-μL volumes with equal volumes of the viral

suspension containing 100 $TCID_{50}$ of cytopathic Iowa strain of IBR virus and cytopathic Oregon-C24V strain of BVD virus. After 1 h of incubation at 37 °C, a suspension of an embryonic bovine trachea (EBTr) cell strain in Eagle's minimal essential medium (EMEM), with 10% fetal bovine serum (FBS), was added to make up a volume of 100 μL. Each test included a back titration of the virus, positive and negative serum control and cell culture control. The results were evaluated after 5 d of incubation at 37 °C in 5% CO_2 in air.

Virus neutralization antibody titres were expressed as the reciprocal of the 50% protection end point, calculated by the Spearman-Kärber method. Sera with a titre $-\log_{10} SNT_{50}$ 0,9 or greater to IBR virus and $-\log_{10} SNT_{50}$ 1,0 or greater to BVD virus were considered positive.

All the dairy cows in this study were examined vaginally and per rectum for reproductive disorders. Excluding clinical findings, data on abortions and fetal mortality were taken from reproductive anamnesis.

Differences in antibody prevalence between groups with and without reproductive disorders for IBR and BVD viruses were tested by the Chi-square test.

RESULTS

Antibody prevalence for IBR virus was 85.8%, and for BVD virus it was 79.2% in the tested cows from all dairy farms.

Table 1 presents antibody prevalence for these viruses for each dairy farm. On the basis of the results we are able to say that the presence of antibodies to IBR and BVD viruses at these farms was approximately equal.

Table 1. Prevalence of IBR and BVD seropositive cows at 4 different dairy farms

Farms	IBR-positive cows n	%	BVD-positive cows n	%
Farm A	44	81.5	42	77.8
Farm B	37	92.5	33	82.5
Farm C	12	85.7	11	78.6
Farm D	10	83.0	9	75.0

Presence of IBR and BVD in dairy cows was considered in relation to their reproductive status (Table 2). In the group of cows that proved positive for IBR and BVD viruses, a statistically significant difference ($P<0.01$) was observed between cows with reproductive disorders and those without reproductive disorders.

Table 2. The number and percentage of cows that tested positive or negative for antibodies to either a single virus and to both viruses, in relation to reproductive disorders

	n	%	Cows with reproductive disorders n (%)	Cows without reproductive disorders n (%)
Positive IBR+BVD	81	67.5	59 (80.8)[a]	22 (46.8)[a]
Positive IBR	13	10.8	6 (8.2)	7 (14.9)
Positive BVD	7	5.8	4 (5.5)	3 (6.4)
Negative IBR+BVD	19	15.9	4 (5.5)	15 (31.9)
Total	120	100	73 (60.8)	47 (39.2)

[a]Statistically significant difference between percentage proportions ($P<0.01$).

The likelihood ratio test for the influence of IBR and BVD viruses on reproductive disorders was significant (likelihood ratio $\chi^2 = 3.95$, df = 1; $P<0.05$; Table 2).

A reciprocal relation between IBR and BVD positive cows with reproductive disorders and cows without reproductive disorders on single dairy farms bring up Table 3.

Table 3. Presence of antibodies to IBR and BVD viruses in sera of cows with and without reproductive disorders at each single dairy farm

	Total		Cows with reproductive disorders		Cows without reproductive disorders	
Location	No. of IBR+BVD positive/n tested	%	No. of IBR+BVD positive/n tested	%	No. of IBR+BVD positive/n tested	%
Farm A	35/54	64.8	24/31	77.4	11/23	47.8
Farm B	30/40	75.0	23/26	88.5	7/14	50.0
Farm C	9/14	64.3	6/9	66.7	3/5	60.0
Farm D	7/12	58.3	6/7	85.7	1/5	20.0

Comparison of reproductive disorders in cows from the 4 dairy farms are presented in Table 4. These reproductive disorders occurred at similar rates among the farms. The most frequently occurring reproductive disorder at all 4 dairy farms was that of repeat breeding, which affected 15 to 35% of the cows. Small percentages of stillbirths, perimetritis and atrophic ovaries were also found, although these disorders were not present at all the farms.

Table 4. Frequency of reproductive disorders in the tested cows at 4 dairy farms

Reproductive disorders	Farm A	Farm B	Farm C	Farm D
Retention of placenta	21%	26%	21%	22%
Aciklia	16%	18%	25%	17%
Cystic ovaries	9%	15%	12.5%	6%
Atrophic ovaries	2%	4%	0%	5.5%
Repeat breeding	35%	15%	21%	33%
Stillbirths	2%	0%	0%	11%
Abortions	5%	11%	12.5%	0%
Endometritis	7%	11%	8%	5.5%
Perimetritis	2%	0%	0%	0%

DISCUSSION

The percentage of seropositive animals in this study was 79.2% to BVD and 85.8% to IBR virus. Cows in this study were not vaccinated against IBR and BVD, and showed no symptoms of disease at the time of examination. In a similar case in nonvaccinated cows, antibodies to IBR virus were present in 34.9% of the animals, while BVD virus was present in 68% of the cases (10).

In an earlier study (21), seroprevalence of BVD and IBR at 12 dairy farms was 100% for herd seroprevalence, while individual seroprevalence was 50.9% for BVD and 41.0% for IBR.

Our results show that the prevalence of IBR and BVD in dairy cows with reproductive disorders was extremely high (80.8%). There was a significant statistical difference in the number of IBR- and BVD-positive cows between the group with reproductive disorders and the group without disorders, suggesting that simultaneous infection with IBR and BVD viruses may have a greater influence on the occurrence of reproductive disorders in dairy cows than monoinfection with either IBR or BVD virus. Similar results, with 93% seropositive to BVD and 40% to IBR virus, have been reported by others (2). Microbiological and serological investigations of uteri and cervix rinses from cows infected with both BVD and IBR showed a high prevalence of BVD and IBR viruses (5).

On the studied farms, the average rate of abortions was 7.13%, of endometritis 7.9%, and of stillbirths 3.3%; while the retention of placentas was 23%. Data on the effect of BVD infection on pregnancy rates, stillbirths, mortality of neonatal calves and the size of new-born calves in persistently infected cattle was evaluated in 8 herds (14). At the time of conception, a significant drop in pregnancy rate to about half the herd average was also found. Moreover, new-born calves were significantly smaller than normal calves (14). In another study (8), experimental or natural infection of 4 heifers with BVD virus in early pregnancy (from 29 to 41 d) resulted in fetal death; 2 heifers aborted and the fetus was resorbed in two other cases. It is doubtful that early pregnancies are terminated by BHV-1 infection, although one study (13) reported a significant

decrease in the number of successful inseminations in seronegative and seropositive dairy cows. The incidence of abortions between BVDV-infected and control groups of dairy cows, was statistically significantly different, while the number of stillbirths, weak-born calves and congenital anomalies was not. The percentage of nonreturns, average number of inseminations per cow, and calving intervals showed a trend toward improvement in the BVDV group (9).

In conclusion our results indicate that the interaction between IBR and BVD viruses could increase reproductive disorders in dairy cows, and thus greatly affect reproductive management practices to implement eradication program for IBR and BVD. The use of marker vaccines offers good prospects for the eradication of herpesvirus infections (15). Use of vaccinations against IBR and BVD in dairy herds were shown to drop the rate of abortion rate from 30 to 4% (16).

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