Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
PREVALENCE OF ANTIBODIES TO IBR AND BVD VIRUSES IN DAIRY COWS WITH REPRODUCTIVE DISORDERS

N. Biuk-Rudan, S. Cvetnić, J. Madić and D. Rudan

1Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine
University of Zagreb, Zagreb, Croatia
2Centre for Reproduction and Breeding of Animals
Zagreb, Croatia

Received for publication: May 15, 1998
Accepted: September 14, 1998

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.

O 1999 by Elsevier Science Inc.

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...
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
occurred over the few last years. We have tested for one group of cows with reproductive
problems 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.

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.

| Farms   | IBR-positive cows | BVD-positive cows |   |
|---------|-------------------|-------------------|---|
|         | n   | %    | 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

| 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
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).

REFERENCES

1. Alenius S, Niskanen 1, Juntti N, Larsson B. Bovine Coronavirus as the causative agent of winter dysentery: serological evidence. Acta Vet Scand 1991; 32: 163-170.
2. Allegri G, Cavirani S, Bottarelli E. Anticorpi neutralizzanti i virus rinotracheite infettiva (IBR) e diarrhoea virale (BVD) nel siero di bovine di allevamenti con ipoefertilita a carattere enzootico. Archo Vet Ital 1985; 36: 174-178.
3. Ames TR. The causative agent of BVD; its epidemiology and pathogenesis. Vet Med 1986; 81: 848-869.
4. Barber DML, Nettleton PF, Herring JA. Disease in a dairy herd associated with the introduction and spread of bovine virus diarrhoea virus. Vet Rec 1985; 117: 459-464.
5. Bottarelli A, Cavirani S, Lucidi E, Allegri G, Zaccarini D. Reperti microbiologici e serologici da bovine appartenenti ad aziendi con scarsa efficienza riproduttiva. Archo Vet Ital 1988; 39: 97-103.
6. Brownlie J, Nuttal PA, Stott EJ, Taylor G, Thomas LH. Experimental infection of calves with two strains of bovine virus diarrhoea virus: certain immunological reactions. Vet Immunol Immunopath 1980; 1: 371-378.
7. Brownlie J. The pathogenesis of bovine virus diarrhoea virus infections. Rev Sci Tech Off Int Epiz 1990; 9: 43-59.
8. Carlsson U, Fredriksson G, Alenius S, Kindahl H. Bovine virus diarrhoea virus, a cause of early pregnancy failure in the cow. J Vet Med 1989; 36: 15-23.
9. Fredriksen B, Odegaard SA, Loken T. The effect of bovine virus diarrhoea virus on reproduction in recently infected Norwegian dairy herds. Acta Vet Scand 1998; 39: 99-108.
10. Fulton RW, Seger CL. Infectious bovine rhinotracheitis, bovine viral diarrhoea and parainfluenza-3 viral antibodies in Louisiana cattle. Bov Pract 1982; 17: 63-65.
11. Gibbs EPJ, Rweyemamu MM. Bovine herpesviruses. Part I. Bovine herpesvirus 1. Vet Bull 1977; 47: 317-343.
12. Grahn TC, Fahning ML, Zemjanis R. Nature of early reproductive failure caused by bovine viral diarrhoea virus. J Am Vet Med Assoc 1984; 185: 429-432.
13. Hage JJ, Schukken YH, Dijkstra T, Barkema HW, van Valkengoed PH, Wentink GH. Milk production and reproduction during a subclinical bovine herpesvirus 1 infection on a dairy farm. Prev Vet Med 1998; 34: 97-106.
14. Houe H, Meyling A. Surveillance of cattle herds for bovine virus diarrhoea virus (BVDV)-infection using data on reproduction and calf mortality. Arch Virol 1991; 3: 157-164.
15. Kaashoek MJ, Moerman A, Madić J, Rijsewijk FAM, Quak J, Gielkens ALJ, Van Oirschot JT. A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. Vaccine 1994; 12: 439-444.
16. Marcus S, Avraham A, Zacks M. A clinical and serological survey of dairy herds vaccinated against IBR, BVD and PI3 infections. Isr J Vet Med 1992; 47: 61-66.
17. Miller MJ, Van Der Maaten JM. Reproductive tract lesions in heifers after intrauterine inoculation with infectious bovine rhinotracheitis virus. Am J Vet Res 1984; 45: 790-794.
18. Miller MJ, Van Der Maaten JM. Experimentally induced infectious bovine rhinotracheitis virus infection during early pregnancy: Effect on the bovine corpus luteum and conceptus. Am J Vet Res 1986; 47: 223-228.
19. Miller MJ. The effects of IBR virus infection on reproductive function on cattle. Vet Med 1991; 86: 95-98.
20. Potgieter LND, McCracken MD, Hopkins FM, Walker RD, Guy JS. Experimental production of bovine respiratory tract disease with bovine viral diarrhoea virus. Am J Vet Res 1984; 45: 1582-1585.
21. Riedemann S, Reinhardt G, Tadich N, Aquilar M, Aquilar R, Montecinos MI, Miranda JC. Seroprevalencia de VDVB, VHB-1, PI-3 y VRSB en 12 predios lecheros de la Provincia de Valdivia, Chile. Arch Med Vet 1996; 28: 121-124.
22. Straub OC. Infectious Bovine Rhinotracheitis Virus. In: Dinter Z, Morein B (eds), Virus Infections of Ruminants. Amsterdam: Elsevier, 1990; 71-108.
23. Wentink GH, van Oirschot JT, Verhoeff J. Risk of infection with bovine herpes virus 1 (BHV1): a review. Vet Quart 1993; 15: 30-33.