



ELSEVIER

Veterinary Microbiology 64 (1999) 253–257

**veterinary
microbiology**

Prevalence of bovine viral diarrhea virus infection in bulls in artificial insemination centers in Poland

M.P. Polak^{*}, J.F. Zmudzinski

National Veterinary Research Institute, Al. Partyzantow 57, 24-100, Pulawy, Poland

Abstract

This study was directed at the evaluation of the prevalence of bovine viral diarrhea virus (BVDV) infection in bulls in artificial insemination centers. Both serological and virological examinations were performed. Blood samples were tested in micro-seroneutralization test for BVDV antibodies. Virus isolation was performed in cell culture and BVDV antigen was detected in an indirect immunofluorescence assay with monoclonal antibodies. One hundred and seventy-five serum samples and 219 whole blood samples for virus isolation were tested. Neutralizing antibodies were found in 86% of the bulls. Persistent infection (PI) was detected in 0.9% of the analyzed blood samples. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Bovine viral diarrhea virus; BVDV; Persistent infection; Seroprevalence; Bulls; Virus isolation

1. Introduction

Bovine viral diarrhea virus (BVDV) is a common cattle pathogen with world-wide distribution (Baker, 1987; Houe, 1995). This widespread occurrence makes BVDV infection economically significant (Duffel et al., 1986; Houe, 1995). In Poland 83% of cows, calves and heifers carry antibodies against BVDV (Polak and Zmudzinski, 1995). The main source of BVDV in the herd is a PI animal, which carries and sheds the virus for life (Coria and McClurkin, 1978). Also acutely infected cattle shed virus in excretions and secretions during viremia (Houe, 1995). BVDV is also excreted in semen in both acutely and persistently infected bulls (Whitmore et al., 1978; Meyling and Jensen, 1988; Paton et al., 1989; Kirkland et al., 1991, 1994; Kommisrud et al., 1996). Use of BVDV-infected semen may lead to acute infection in cows and acute or persistent infection in

^{*} Corresponding author. Tel.: +48-81-886-30-51 ext. 115; fax: +48-81-886-25-95; e-mail: ppolak@piwet.pulawy.pl

their progeny. Also, reduced conception rates in seronegative cows when using BVDV-contaminated semen can be observed (McClurkin et al., 1979; Whitmore et al., 1981). Moreover, animals acutely or persistently infected with BVDV may look healthy, and clinical examination will not provide any suspicion of BVDV infection (Coria and McClurkin, 1978; Cutlip et al., 1980; Barber et al., 1985). Therefore, bulls should be tested, and only virus-negative animals should be introduced into artificial insemination (AI) centers. Serological and virological surveillance of BVDV infection in bulls in AI centers is fundamental for the prevention of virus transmission under modern breeding conditions. The objective of the study was to assess the prevalence of infection in bulls at AI centers in Poland on the basis of serological and virological examination of blood samples.

2. Material and methods

One hundred and seventy-five serum samples were used for micro-seroneutralization (MSN) and 219 samples of whole blood were used for virus isolation (VI). MSN test was performed with 100 TCID₅₀ of NADL reference strain in Madin Darby Bovine Kidney (MDBK) cell line free from pestiviral contamination, grown in minimal essential medium (MEM) supplemented with 5% fetal equine serum (FES). 50 µl of each dilution of serum was mixed with 50 µl of 100 TCID₅₀ of the NADL reference strain. Each serum was tested in triplicate. The titer was read after a 5-day incubation period and expressed as the reciprocal of the highest dilution inhibiting cytopathic effect in triplicate wells. The dilution range was run from 1 : 2 to 1 : 512.

A primary testicle cell line free from pestiviral infection grown in MEM supplemented with 2% FES was used for virus isolation. The monolayer was inoculated with the material obtained as a buffy coat with 1 h adsorption at 37°C, 4% CO₂. After 2 days of incubation, additional two blind sub-passages were made. Cells from the third sub-passage were trypsinized, placed on teflon-covered glass slides and allowed to adhere for 3 h followed by fixation in cold acetone for 20 min. Monoclonal antibodies (MAbs) to the E2/gp53 protein (kindly provided by Dr. Ruben Donis, University of Nebraska, Lincoln, USA) were used in indirect immunofluorescence assay. Anti-mouse IgG labeled with fluorescein isothiocyanate (IgG/FITC) conjugate was used as the second antibody. Incubation time for the MAbs and the conjugate was 60 min in 37°C, 4% CO₂. After counterstaining with 0.01% Evan's Blue, the slides were mounted in buffered glycerin and cytoplasm fluorescence was observed in a microscope under ultra violet light. The animals positive in the first testing were re-tested at least 3 weeks later using the same technique of virus isolation.

3. Results

Serological screening showed that 86% of the tested bulls carried antibodies against BVDV with titers ranging from 2 to 512. The most frequent titer was four, which was found in 25% of the positive samples. Virus isolation was successful in five (2.3%) of the

219 samples submitted for testing. Persistent infection was confirmed in two bulls (0.9%). Serum neutralization titers in those animals were 32 and 64, respectively.

4. Discussion

Transmission of BVDV within the herd is maintained mainly by direct contact with animals persistently and acutely infected with this pathogen. The only method of eliminating the BVDV infection from the herd so far has been the removal of PI animals, which are the main source of the virus (Bitsch, 1995). These animals are rarely encountered. The prevalence of PI animals in cattle populations ranges between 0.5 and 2% (Bolin et al., 1985; Alenius et al., 1986; Howard et al., 1986; Houe and Meyling, 1991; Houe et al., 1995; Frey et al., 1996). Lack of clinical signs of the BVDV infection in most cases calls for laboratory diagnosis as the only way of detection of infected animals. This is particularly important in bulls in AI centers.

In herds with PI individuals, the percentage of seropositive animals is normally high. In our study, the high percentage of BVDV antibody-positive bulls shows that the infection with this virus is common. This is in agreement with data from other countries around the world where up to 90% of cattle are seropositive for BVDV (Ernst et al., 1983; Houe, 1995). In this study serum neutralization titers against the reference NADL strain were in the range from 2 to 512. The most frequent titers were between 2 and 8, comprising 68% of positive samples (Fig. 1). All analyzed samples came from bulls at least 6 months old; therefore, the presence of maternal antibodies can be excluded. Therefore, we believe that these low titers are the result either of a new ongoing infection, or the infecting strains of BVDV differ antigenically from the reference NADL strain used in the study. The last hypothesis has been supported by a comparative study with a panel of MABs.

Three of five virus-positive samples were positive only in the first testing. This confirms that they have come from transiently infected and viremic bulls. Such animals

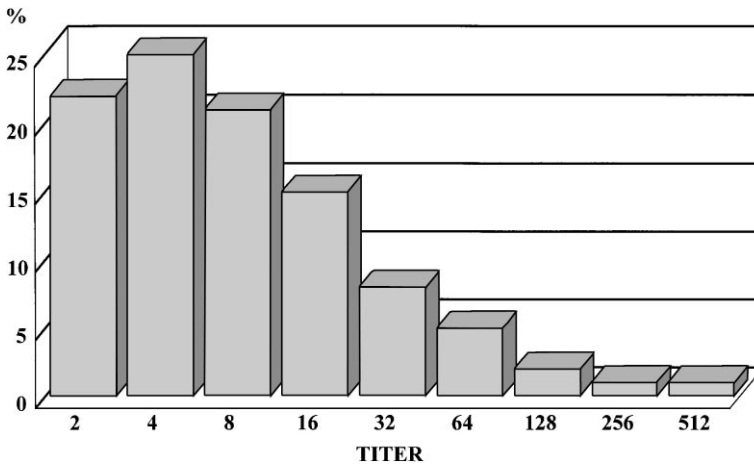


Fig. 1. Frequency of distribution of VN titers in 175 serum samples from AI bulls.

should also be considered as the transient source of the virus although the amount of the pathogen excreted is much lower than in the case of PI animals. Two bulls (0.9%) were positive in the second testing for virus isolation, which confirms the state of persistent infection. Howard et al. (1990) showed 12 (0.78%) of 1538 bulls in AI centers to be persistently infected.

In most cases PI animals are found among seronegative animals existing in the herd with high prevalence of seropositive animals (Houe, 1992). Such animals are immunotolerant and do not produce antibodies against persisting virus. In our study two PI animals had rather high antibody titers, that is, 32 and 64. With the commonly used serological approach when selecting seronegative animals for testing for persistent infection, these two bulls would have been missed. We presume that these bulls must have been infected post-natally with a virus strain antigenically different from the persisting strain of BVDV, and that seroconversion was the result of this post-natal infection.

5. Conclusions

Widespread occurrence of BVDV infection in bulls in AI centers in Poland was confirmed by serological screening. The percentage of PI animals was found to be within the range typical for other countries. Surprisingly high neutralizing antibody titers against the NADL reference strain were detected in PI animals.

References

- Alenius, S. et al., 1986. Frequency of bovine viral diarrhoea virus infection in Sweden among heifers selected for artificial insemination. In: Proc. 14th World Congress on Diseases of Cattle, pp. 204–207.
- Baker, J.C., 1987. Bovine viral diarrhoea virus: A review. *J. Am. Vet. Med. Assoc.* 190, 1449–1458.
- Barber, D.M.L., Nettleton, P.F., Herring, J.A., 1985. Disease in a dairy herd associated with the introduction and spread of bovine virus diarrhoea virus. *Vet. Rec.* 117, 459–464.
- Bitsch, V., 1995. Control of bovine viral diarrhoea virus infection without vaccines. *Vet. Clin. North Am.: Food Animal Pract.* 11, 627–640.
- Bolin, S.R., McClurkin, A.W., Coria, M.F., 1985. Frequency of persistent bovine viral diarrhoea virus infection in selected cattle herds. *Am. J. Vet. Res.* 46, 2385–2387.
- Coria, M.F., McClurkin, A.W., 1978. Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhoea virus. *J. Am. Vet. Med. Assoc.* 172, 449–451.
- Cutlip, R.C., McClurkin, A.W., Coria, M.F., 1980. Lesions in clinically healthy cattle persistently infected with the virus of bovine viral diarrhoea-glomerulonephritis and encephalitis. *Am. J. Vet. Res.* 41, 1938–1941.
- Duffel, S.J., Sharp, M.W., Bates, D., 1986. Financial loss resulting from BVD-MD virus infection in a dairy herd. *Vet. Rec.* 118, 38–39.
- Ernst, P.B., Baird, J.D., Butler, D.G., 1983. Bovine viral diarrhoea: An update. *Compend. Contin. Educ. Pract. Vet.* 5, S581–S589.
- Frey, H.-R., Flebbe, U., Liess, B., 1996. Prävalenz und klinische Symptomatik persistenter BVD-Virusinfektionen in Rinderbeständen Niedersachsens. *Der prakt. Tierarzt* 77, 49–52.
- Houe, H., 1995. Epidemiology of bovine viral diarrhoea virus. *Vet. Clin. North Am.: Food Animal Pract.* 11, 521–547.
- Houe, H., 1992. Serological analysis of a small herd sample to predict presence or absence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy herds. *Res. Vet. Sci.* 53, 320–323.

- Houe, H., Meyling, A., 1991. Prevalence of bovine virus diarrhoea (BVD) in 19 Danish dairy herds and estimation of incidence of infection in early pregnancy. *Prev. Vet. Med.* 11, 9–16.
- Houe, H., Baker, J.C., Maes, R.K., Wuryastuti, H., Wasito, R., Ruegg, P.I., Lloyd, J.W., 1995. Prevalence of cattle persistently infected with bovine viral diarrhoea virus in 20 dairy herds in two counties in central Michigan and comparison of prevalence of antibody-positive cattle among herds with different infection and vaccination status. *J. Vet. Diagn. Invest.* 7, 321–326.
- Howard, T.H., Bean, B., Hillman, R., Monke, D.R., 1990. Surveillance for persistent bovine viral diarrhoea virus infection in four artificial insemination centers. *J. Am. Vet. Med. Assoc.* 196, 1951–1955.
- Howard, C.J., Brownlie, J., Thomas, L.H., 1986. Prevalence of bovine virus diarrhoea virus viraemia in cattle in the UK. *Vet. Rec.* 119, 628–629.
- Kirkland, P.D., MacIntosh, S.G., Mole, A., 1994. The outcome of widespread use of semen from a bull persistently infected with pestivirus. *Vet. Rec.* 135, 527–529.
- Kirkland, P.D., Richards, S.G., Rothwell, J.T., Stanley, D.F., 1991. Replication of bovine viral diarrhoea virus in the bovine reproductive tract and excretion of virus in semen during acute and chronic infections. *Vet. Rec.* 128, 587–590.
- Kommisrud, E., Vatn, T., Lang-Ree, J.R., Lrken, T., 1996. Bovine virus diarrhoea virus in semen from acutely infected bulls. *Acta Vet. Scand.* 37, 41–47.
- McClurkin, A.W., Coria, M.F., Cutlip, R.C., 1979. Reproductive performance of apparently healthy cattle persistently infected with bovine viral diarrhoea virus. *J. Am. Vet. Med. Assoc.* 174, 1116–1119.
- Meyling, A., Jensen, A.M., 1988. Transmission of bovine virus diarrhoea virus (BVDV) by artificial insemination (AI) with semen from a persistently infected bull. *Vet. Microbiol.* 17, 97–105.
- Paton, D.J., Goodey, R., Brockman, S., Wood, L., 1989. Evaluation of the quality and virological status of semen from bulls acutely infected with BVDV. *Vet. Rec.* 124, 63–64.
- Polak, M.P., Zmudzinski, J., 1995. Prevalence of bovine viral diarrhoea virus in the cattle population of Poland. In: *Proc. 3rd Congress Europ. Soc. Vet. Virol., Immunobiology of Viral Infections, Interlaken, Switzerland, 4–7 September 1994*, pp. 309–310.
- Whitmore, H.L., Gustafsson, B.K., Havareshti, P., Duchateau, A.B., Mather, E.C., 1978. Inoculation of bulls with bovine virus diarrhoea virus: Excretion of virus in semen and effects on semen quality. *Theriogenology* 9, 153–163.
- Whitmore, H.L., Zemjamis, R., Olson, J., 1981. Effect of bovine viral diarrhoea virus on conception in cattle. *J. Am. Vet. Med. Assoc.* 178, 1065–1067.