Research in Veterinary Science 100 (2015) 297–298

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Short communication: Transmission of border disease virus to seronegative cows inseminated with infected semen

U. Braun [a,*], S. Frei [a], M. Schweizer [b], R. Zanoni [b], F. Janett [a]

[a] Department of Farm Animals, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland
[b] Institute of Virology and Immunology, Länggass-Strasse 122, CH-3001 Bern, Switzerland

ARTICLE INFO

Article history:
Received 23 August 2014
Accepted 22 March 2015

Keywords:
Cattle
Border disease
Semen
Insemination
Seroconversion
BVD virus
Pestivirus

ABSTRACT

The goal of this study was to investigate the transmissibility of border disease (BD) virus to seronegative cows via artificial insemination with cryopreserved semen from a bull persistently infected with BD virus. Five pestivirus naive cows were inseminated with BD virus-infected semen. Blood was collected for detection of pestivirus antibody by means of an ELISA on day 0 (day of insemination) and then every 7 days until day 56, at which time a serum neutralisation test (SNT) for differentiation of BD and BVD virus was carried out. Seroconversion was first noticed in two cows on day 14, in two cows on day 21 and in one cow on day 28. In the SNT, all cows had distinctly positive titres against BD virus. Therefore, BD virus is readily transmitted by infected semen, but none of the cows conceived, most likely because of poor semen quality.

Border disease (BD) virus was transmitted in semen from an infected ram to ewes after natural breeding (Gardiner and Barlow, 1981), and bovine virus diarrhoea (BVD) virus was transmitted via artificial insemination to seronegative cows (Meyling and Jensen, 1988), demonstrating that semen can be a source of pestivirus infection. The goal of this study was to determine whether BD virus can be transmitted to seronegative cows through infected semen. Semen used in the study was obtained from a Brown Swiss × Limousin bull persistently infected (pi) with a BD virus strain (BD "Switzerland"; Peterhans et al., 2010). Postmortem examination of the pi bull revealed mild orchitis and testicular degeneration and the presence of BD virus in the testes. Collection, morphological and virological examination and cryopreservation of the semen have been described in detail (Frei, 2014) as well as the results of radiographic examination of the extremities of the pi bull (Frei et al., 2014, animal No. 3).

Six clinically healthy, non pregnant Brown Swiss cows, aged 3.0 ± 0.4 years, and tested negative for pestivirus antigen, were purchased for the study. The study period was divided into an acclimation and an infection phase. During acclimation for 48 ± 8 days, the cows were kept in quarantine. The cows were tested negative for pestivirus antibody in an ELISA before and after acclimation. Five cows were inseminated with semen from the pi bull three times, on two consecutive days, following estrus synchronisation. The semen had a high virus titre at 2.51×10^8 (50% tissue culture infective dose [$TCID_{50}$]/ml) and 1.44×10^6 ($TCID_{50}/10^6$ sperm cells). Virus load of semen did not correlate with sperm count in different ejaculates, suggesting that the virus was mainly located in the seminal fluid (Kirkland et al., 1991). In fresh semen, progressive sperm motility was low (23%) and morphological sperm abnormalities were abundant (90%). One cow was not inseminated and served as a control. The cows were checked for pregnancy using transrectal ultrasonography 28 days after the last insemination.

The infection phase began at the time of first insemination (day 0), and lasted 56 days. Blood was collected for evaluation of pestivirus antibody on day 0 of the infection phase and then every 7 days until day 56. On day 56, samples were also analysed by serum neutralisation test (SNT) to differentiate between BD and BVD virus. An "in-house" antibody ELISA (Canal et al., 1998) was used for pestivirus antibody detection in serum. The optical density (OD) was expressed as percentage of the OD of a standard serum; relative OD readings between 20% and 30% were considered indeterminate and those >30% were considered positive. SNT was used to identify the pestivirus specificity of the antibodies (Danuser et al., 2009; the BD virus type that was isolated from the bull calf was used instead of the Moredun type). As cross reactions between BVD and BD viruses are common based on their genetic relationship (Becher et al., 2003), only BD virus antibody titres at least four times higher than the BVD virus antibody titres were considered significantly higher (Braun et al., 2013; Danuser et al., 2009).

All animals remained clinically inapparent with the exception of increased temperature (>39.0 °C) on 1 or 2 days in 2 of 5

* Corresponding author. Department of Farm Animals, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland. Tel.: +41 1 6358241; fax: +41 1 6358904.
E-mail address: ubraun@vetclinics.uzh.ch (U. Braun).

http://dx.doi.org/10.1016/j.rvsc.2015.03.027
0034-5288/

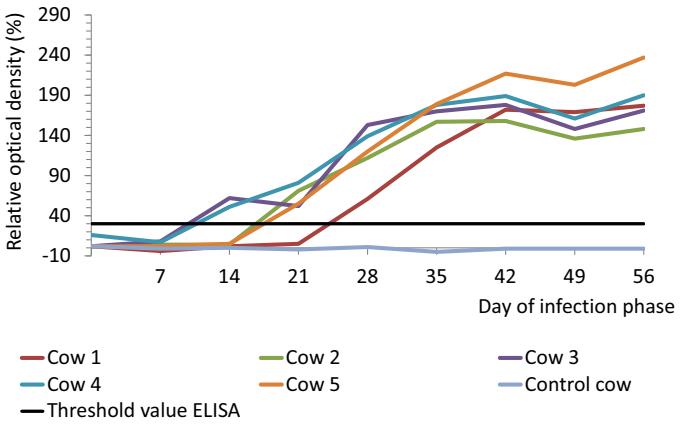

Fig. 1. Relative optical density in the ELISA for pestivirus antibody expressed as percentage of the optical density of a standard serum in the control cow and five cows after artificial insemination with BD virus-infected semen.

Table 1
Relative values in the ELISA for pestivirus antibody, SNT titres and quotient of BD and BVD virus antibody titres in 5 cows inseminated with BD virus-infected semen and in the control cow at the end (day 56) of the infection phase.

Cow	OD value (%)	SNT BD virus	SNT BVD virus	Quotient of BD and BVD virus antibody titres
1	177	431	27	16
2	148	304	11	28
3	171	215	16	13
4	190	256	11	23
5	237	512	27	19
Control	−1	<6	<6	NA

NA, not applicable.

inseminated cows and in the control cow, but only late after day 48. All of the five inseminated cows remained seronegative to day 7 after insemination (Fig. 1). Seroconversion with relative OD values >30% (62 and 51%) was first noted in cows 3 and 4 on day 14, followed by cows 2 (71%) and 5 (55%) on day 21 and cow 1 (61%) on day 28. The relative OD value increased markedly until day 42 in all five cows, ranging from 148 to 237% at the last examination (Table 1). The control cow remained seronegative throughout the study. SNT on day 56 revealed that the five inseminated cows were positive for BD virus antibody with titres of 215–512. In contrast, the same five cows had very low antibody titres against BVD virus (11–27), and the quotient of BD and BVD virus antibody titres was >4 (13–28), which clearly indicated antibody production in response to BD virus infection. SNT in the control cow was negative for both pestiviruses (<6). All of the five cows came into estrus 20–23 days after the last insemination. Transrectal ultrasonography 28 days after insemination revealed that none of the cows was pregnant.

Studies on the response of seronegative cows to insemination with BD virus-infected semen are lacking. The present study showed that all five cows underwent seroconversion by day 28 post insemination and had antibody titres that progressively increased until day 42. In the same time period, the control cow remained seronegative despite being in close contact with the infected cows. This strongly suggests that seroconversion occurred in response to BD virus-infected semen and that the different times of seroconversion are not the result of horizontal virus transmission. Similar increases in antibody titres were seen in seronegative ewes that were artificially inseminated with BD virus-infected sperm or bred by a persistently infected ram; antibodies against BD virus were detected in all ewes within 10–30 days (Gardiner and Barlow, 1981). Seronegative cows seroconverted within 14 days after artificial insemination with semen from a bull persistently infected with BVD virus, and cows inseminated with virus-free semen remained seronegative despite being in contact with the infected cows (Meyling and Jensen, 1988).

In the five inseminated cows, neutralising antibody titres were high for BD virus and low for BVD virus 56 days after insemination. The low antibody titres against BVD virus were attributed to partial cross-neutralisation within the pestivirus genus. The antibody titres against BD virus were 13–28 times higher than the titres against BVD virus, and all the five cows were thus considered to be infected with BD virus. However, none of the inseminated cows conceived, probably due to the poor quality of the infected semen. Therefore, BD virus can readily be transmitted by infected semen, but the direct role of BD virus in the rate of conception remains to be investigated.

Acknowledgements

We thank Dr. Josef Risi and Dr. Toni Linggi, Veterinary Office of the founding cantons, for provision of the pi BDV calf, Sarun Keo for semen evaluation and cryopreservation, the laboratory technicians of the Institute of Veterinary Virology for the examination of the samples and Hanspeter Müller for taking care of the animals.

References

Becher, P., Avalos Ramirez, R., Orlich, M., Cedillo Rosales, S., König, M., Schweizer, M., et al., 2003. Genetic and antigenetic characterization of novel pestivirus genotypes: implications for classification. Virology 311, 96–104.
Braun, U., Bachofen, C., Schenk, B., Hässig, M., Peterhans, E., 2013. Investigation of border disease and bovine virus diarrhoea in sheep from 76 mixed cattle and sheep farms in eastern Switzerland. Schweizer Archiv für Tierheilkunde 155, 293–298.
Canal, C.W., Strasser, M., Hertig, C., Masuda, A., Peterhans, E., 1998. Detection of antibodies to bovine viral diarrhoea virus (BVDV) and characterization of genomes of BVDV from Brazil. Veterinary Microbiology 63, 85–97.
Danuser, R., Vogt, H.R., Kaufmann, T., Peterhans, E., Zanoni, R., 2009. Seroprevalence and characterization of pestivirus infections in small ruminants and new world camelids in Switzerland. Schweizer Archiv für Tierheilkunde 151, 109–117.
Frei, S., 2014. Übertragung des Border-Disease-Virus von einem persistent infizierten Rind auf seronegative Rinder durch Kontaktinfektion und virushaltiges Sperma. DrMedVet Thesis, Vetsuisse Faculty, University of Zurich.
Frei, S., Braun, U., Dennler, M., Hilbe, M., Stalder, H.P., Schweizer, M., et al., 2014. Border disease in persistently infected calves: radiological and pathological findings. Veterinary Record 174, 170.
Gardiner, A.C., Barlow, R.M., 1981. Vertical transmission of Border disease infection. Journal of Comparative Pathology 91, 467–470.
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. Veterinary Record 128, 587–590.
Meyling, A., Jensen, A.M., 1988. Transmission of bovine virus diarrhoea virus (BVDV) by artificial insemination (AI) with semen from a persistently-infected bull. Veterinary Microbiology 17, 97–105.
Peterhans, E., Bachofen, C., Stalder, H.P., Schweizer, M., 2010. Cytopathic bovine viral diarrhea viruses (BVDV): emerging pestiviruses doomed to extinction. Veterinary Research 41, 44.