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

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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 naïve 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 seven 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.

Keywords: Cattle; Border disease; Semen; Insemination; Seroconversion; BVD virus; Pestivirus

Border disease (BD) virus was transmitted in semen from an infected ram to ewes after natural breeding (Gardiner and Barlow, 1981), and bovine virus diarrhea (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 x 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

48 semen have been described in detail (Frei, 2014) as well as the results of radiographic
49 examination of the extremities of the pi bull (Frei et al., 2014, animal No. 3).
50 Six clinically healthy, non pregnant Brown Swiss cows, aged 3.0 ± 0.4 years, and tested
51 negative for pestivirus antigen, were purchased for the study. The study period was divided into
52 an acclimation and an infection phase. During acclimation for 48 ± 8 days, the cows were kept in
53 quarantine. The cows tested negative for pestivirus antibody in an ELISA before and after
54 acclimation. Five cows were inseminated with semen from the pi bull three times, on two
55 consecutive days, following estrus synchronisation. The semen had a high virus titre at 2.51 x
56 10^8 (50 % tissue culture infective dose [$TCID_{50}$]/ml) and 1.44 x 10^6 ($TCID_{50}/10^6$ sperm cells).
57 Virus load of semen did not correlate with sperm count in different ejaculates, suggesting that
58 the virus was mainly located in the seminal fluid (Kirkland et al., 1991). In fresh semen,
59 progressive sperm motility was low (23 %) and morphological sperm abnormalities were
60 abundant (90 %). One cow was not inseminated and served as a control. The cows were checked
61 for pregnancy using transrectal ultrasonography 28 days after the last insemination.
62 The infection phase began at the time of first insemination (day 0), and lasted 56 days.
63 Blood was collected for evaluation of pestivirus antibody on day 0 of the infection phase and
64 then every seven days until day 56. On day 56, samples were also analyzed by serum
65 neutralization test (SNT) to differentiate between BD and BVD virus. An "in-house" antibody
66 ELISA (Canal et al., 1998) was used for pestivirus antibody detection in serum. The optical
67 density (OD) was expressed as percentage of the OD of a standard serum; relative OD readings
68 between 20 % and 30 % were considered indeterminate and those > 30 % were considered
69 positive. SNT was used to identify the pestivirus specificity of the antibodies (Danuser et al.,
70 2009; the BD virus type that was isolated from the bull calf was used instead of the Moredun
71 type). As cross reactions between BVD and BD virus are common based on their genetic

72 relationship (Becher et al., 2003), only BD virus antibody titres at least four times higher than
73 the BVD virus antibody titres were considered significantly higher (Danuser et al., 2009, Braun
74 et al., 2013).
75 All animals remained clinically inapparent with the exception of increased temperature (>
76 39.0 °C) on one or two days in 2 of 5 inseminated cows and in the control cow, but only late
77 after day 48. All of the five inseminated cows remained seronegative to day 7 after insemination
78 (Fig. 1). Seroconversion with relative OD values > 30 % (62 and 51 %) was first noted in cows 3
79 and 4 on day 14, followed by cows 2 (71 %) and 5 (55%) on day 21 and cow 1 (61 %) on day
80 28. The relative OD value increased markedly until day 42 in all five cows, ranging from 148 to
81 237 % at the last examination (Table 1). The control cow remained seronegative throughout the
82 study. SNT on day 56 revealed that the five inseminated cows were positive for BD virus
83 antibody with titres of 215 to 512. In contrast, the same five cows had very low antibody titres
84 against BVD virus (11 to 27), and the quotient of BD and BVD virus antibody titres was > 4 (13
85 to 28), which clearly indicated antibody production in response to BD virus infection. SNT in
86 the control cow was negative for both pestiviruses (< 6). All of the five cows came into estrus 20
87 to 23 days after the last insemination. Transrectal ultrasonography 28 days after insemination
88 revealed that none of the cows was pregnant.
89 Studies on the response of seronegative cows to insemination with BD virus-infected
90 semen are lacking. The present study showed that all five cows underwent seroconversion by
91 day 28 post insemination and had antibody titres that progressively increased until day 42. In the
92 same time period, the control cow remained seronegative despite being in close contact with the
93 infected cows. This strongly suggests that seroconversion occurred in response to BD virus-
94 infected semen and that the different times of seroconversion are not the result of horizontal
95 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 ten to 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 to 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.

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147 Figure 1: Relative optical density in the ELISA for pestivirus antibody expressed as
148 percentage of the optical density of a standard serum in the control cow and five cows after
149 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