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Virulence comparison and quantification of horizontal bovine viral diarrhoea virus transmission following experimental infection in calves

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ABSTRACT

Bovine viral diarrhoea virus (BVDV) causes persistent infections by infecting the fetus of susceptible animals during gestation. These persistently infected (PI) animals are important sources of infection. On the contrary, transiently infected (TI) animals are believed to be less important, but transient infections with a severe BVDV-2 strain can spread explosively.

To assess the importance of TI cattle in the epidemiology of BVDV, two experimental infections were performed to determine basic reproduction ratios (R_0). In each experiment three calves were infected via intranasal inoculation and housed together with seven susceptible animals. Two strains isolated in Belgium were used, a virulent BVDV-1b and a virulent BVDV-2a field isolate, resulting in an R_0 of 0.25 (95% CI 0.01; 1.95) and 0.24 (95% CI 0.01; 2.11), respectively. A PI animal was then introduced to the remaining uninfected animals and produced an R of $+\infty$ (95% CI 1.88; $+\infty$). These results support the suggestion that TI animals, compared to PI animals, contribute only a limited amount to BVDV spread. Additionally, the severe clinical symptoms observed in the field with these isolates could not be reproduced during these experiments, suggesting that other factors besides strain virulence influence the clinical manifestations evoked by BVDV.

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Introduction

Bovine viral diarrhoea virus (BVDV) is characterized by a strong genetic diversity and can be divided into two genotypes, BVDV-1 and BVDV-2, each subdivided into subgenotypes (Neill, 2013). Disease severity is likely to be linked to the degree of viraemia (Walz et al., 2001).

Acute BVDV infections of BVDV seronegative cattle result in a transient viraemia of 10–14 days, starting 3 days post-infection (Lanyon et al., 2014). The duration of virus shed by these transiently infected (TI) animals varies considerably and is likely to depend on the virulence of the strain (Bolin and Ridpath, 1992). After 5–7 days of incubation about 70–90% of acute infections are subclinical, associated with only a mild raise in body temperature and leukopenia (Baker, 1995). By infecting cells of the immune system, BVDV evokes an immunosuppression leading to a decreased response to other infectious agents (Chase et al., 2004). BVDV also causes persistent infections by infecting the fetus in early gestation (Peterhans et al., 2010). Once colostrum-derived BVDV antibody titres have declined, these persistently infected (PI) animals continuously shed BVDV in large quantities (Lindberg and Houe, 2005).

The transmission rate of an infectious disease in a population can be expressed by its reproduction ratio, R. A special case is the basic reproduction ratio, R_0, defined as the mean number of new infections arising from one typical infectious case introduced in a fully susceptible population. R has a threshold value equal to 1. This means that an infection may spread when $R > 1$ and will fade out when $R < 1$ (Velthuis et al., 2007). PI animals are very successful virus transmitters (R_0 assumed >1), whereas TI animals are believed to be less important in spreading BVDV (R_0 assumed <1), due to the shorter duration of infection and the intermittent shedding of relatively low amounts of virus (Lindberg and Houe, 2005). However, it has been suggested that transient infections with a severe BVDV-2 strain can spread explosively (Ridpath et al., 2006).

Belgium is characterized by a high cattle density and a high BVDV prevalence; about one-third of the young stock is BVDV-seropositive (Sarrazin et al., 2013). In addition, severe clinical symptoms linked

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to BVDV-1 and BVDV-2 have been described (Letellier et al., 2010; Laureyns et al., 2011a, 2011b, 2013). We hypothesized that TI animals may contribute substantially more to BVDV spread than is generally assumed. The objective of the present study was to estimate R_0 for BVDV transmission by TI cattle following experimental infections using Belgian BVDV field isolates.

Materials and methods

All experiments were approved by the Ethics Committee of the Belgian Veterinary and Agrochemical Research Centre (reference number 120102-01 BOVIDI1, 2 January 2012).

Animals

For each trial 10 Holstein-Friesian calves were selected from BVDV-free herds (no BVDV history despite regular monitoring). The calves were checked twice for the absence of BVDV-RNA and antibodies using real-time RT-PCR (RT-qPCR) and virus neutralization (VN), respectively, as described below. At the time of infection, animals in trials 1 and 2 were aged between 65 and 90 days and between 100 and 128 days, respectively.

Virus isolates

For trial 1, a non-cytopathic (ncp) BVDV-1b strain isolated from a 2-day-old PI animal with spontaneous skin bleeding (Laureyns et al., 2013) was cultivated on Madin-Darby Bovine Kidney (MDBK) cells (one passage). The titre used was 2.6×10^6 tissue culture infective dose/mL ($TCID_{50}$/mL). For trial 2, the ncp BVDV-2a isolate 07/3913 was used (Letellier et al., 2010) after cultivation (two passages). The titre used was 3.7×10^5 $TCID_{50}$/mL.

Experimental design (Table 1)

Trial 1 calves were housed together in a box 3.2×10.0 m on a slatted floor, which was cleaned daily with water. After an acclimatization period of 14 days, three randomly chosen calves were isolated from the other calves and inoculated with BVDV-1b through intranasal inoculation of 5.0×10^6 $TCID_{50}$. After 2 days of separation the inoculated animals were housed with the seven contact animals. When no infectious animals were left, i.e. all blood samples and nasal swabs were negative by RT-qPCR (69 days after study start), all BVDV-seropositive animals were removed, and the trial process was repeated with three of the remaining calves being inoculated with the virus. All 10 calves were slaughtered 38 days after the start of this second phase of the trial (trial 1.2).

For trial 2, housing conditions, acclimatization period and inoculation procedures were identical to trial 1, except that the BVDV-2a strain was used. When no infectious animals were left (56 days after study start), all BVDV-seropositive animals were removed, and the trial process was repeated (trial 2.2) with three of the remaining calves inoculated with the virus. Forty-four days later, a 6-month-old calf persistently infected with a BVDV-1b strain was commingled with all 10 calves from trial 2 (seven of which had seroconverted to BVDV-2a and three of which had remained susceptible) (trial 2.3). The serum viral titre of the PI calf was approximately 2.4×10^4 $TCID_{50}$/mL. All 11 animals were slaughtered 34 days after the introduction of the PI animal.

Clinical examination and sample collection

Clinical characteristics and rectal temperatures were recorded during daily examination using a scoring system adapted from Cortese et al. (1998) and Pardon (2012) (see Appendix: Supplementary material).

Whole blood was collected in EDTA-coated tubes and stored at 4 °C. Serum was collected in tubes with a cloth activator, centrifuged for 10 min at 1800 *g* and stored at –20 °C. Nasal swabs were dipped in 0.5 mL Minimum Essential Medium (MEM, Invitrogen) containing penicillin, gentamicin and amphotericin B and stored at –80 °C.

Virus isolation and determination of viral titre

An in-house haemolysis buffer (0.31 M NH_4Cl, 0.02 M $NaHCO_3$ and 0.63 mM Na_2EDTA) was added to 1.5 mL blood. A pellet of leucocytes was obtained after centrifugation for 10 min at 200 *g*, suspended in 1 mL MEM and used to make 10-fold dilutions. Serial dilutions were inoculated in 96-well microtitre plates containing monolayers of MDBK-cells in MEM, 10% fetal calf serum, L-glutamine and antibiotics.

After incubation for 6 days at 37 °C in humidified air containing 5% CO_2, the supernatant was removed and the monolayers were rinsed with phosphate-buffered saline (PBS) solution, dried overnight at 37 °C and stored at –20 °C for a minimum of 12 h to lyse the cells. MDBK-cells were stained for BVDV-antigen using an immunoperoxidase monolayer assay (IPMA) adapted from Jensen (1981). The monolayers were fixed with 4% formalin, rinsed, overlaid with 100 µL of BVDV polyclonal serum diluted 1/200 and incubated for 1.5 h at 37 °C. Washing and staining procedures were performed (Jensen, 1981), using a rabbit anti-bovine peroxidase conjugate (Sigma-Aldrich) diluted 1/500. Reading was done using a light microscope and viral titre was calculated (Reed and Muench, 1938).

Table 1
Overview and results of the experimental design. The status of a calf at the start and the end of each trial is presented. The days of sample collection are shown on the right.

Trial 1: BVDV-1b transmission through TI animals

Days after first inoculation		Calf										Sampling (days after each inoculation)
		1	2	3	4	5	6	7	8	9	10	
First inoculation												
Start	0	I	I	I	S	S	S	S	S	S	S	0; 2–10: daily; 12–24, 27–31, 34–38: every 2 days; 45
End	69	R	R	R	S	S	S	S	S	S	S	
Second inoculation												
Start	69				I	I	I	S	S	S	S	0; 2–11: daily; 14–18, 21–25: every 2 days; 30
End (slaughter)	107				R	R	R	C	S	S	S	

Trial 2: BVDV-2a transmission through TI animals

Days after first inoculation		Calf											Sampling (days after each inoculation)
		1	2	3	4	5	6	7	8	9	10	PI	
First inoculation													
Start	0	I	I	I	S	S	S	S	S	S	S	–	0; 2–12: daily; 14–18, 21–31: every 2 days; 37; 45; 51
End	56	R	R	R	C	S	S	S	S	S	S	–	
Second inoculation													
Start	56					I	I	I	S	S	S	–	0; 2–4, 7–11, 14–18: every 2 days; 22; 25
End	100					R	R	R	S	S	S	–	
Introduction of PI animal: BVDV-1b transmission through PI animal													
Start	100	R	R	R	R	R	R	R	S	S	S	PI	0; 2; 5; 8; 12; 14; 19; 26; 28; 33
End (slaughter)	134	R	R	R	R	R	R	R	C	C	C	PI	

TI, transiently infected; PI, persistently infected; I, infectious; S, susceptible; C, contact infected; R, recovered.

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