Transient elimination of circulating bovine viral diarrhoea virus by colostral antibodies in persistently infected calves: a pitfall for BVDV-eradication programs?

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ABSTRACT

Infections with bovine viral diarrhoea virus (BVDV) cause substantial economic losses to cattle industries. Rapid detection of persistently BVDV infected (PI) calves is of utmost importance for the efficacy of BVDV control programs. Blood and ear skin biopsy samples are conveniently used for early mass screening of newborns. However, little is known about the impact of colostral antibodies on the outcome of relevant analyses. Here, we rigorously tested a series of samples obtained from five colostrum-fed PI calves from birth until they reached the status of seronegativity for NS3-specific antibodies. We comparatively quantified virus loads in blood samples and dried skin biopsies as detected with BVDV-NS3-, -Erns-capture ELISA and RT-qPCR. Monitoring of NS3-positive leukocytes was done with flow cytometry. Within seven days after colostrum intake, BVDV infected leukocytes disappeared for a three- to eight-week period. Immediately after colostrum ingestion, detectable Erns antigen levels dropped 10–100-fold in biopsy samples and in sera detection of Erns failed for one to two weeks. Virus demonstration in biopsy samples with a NS3-antigen-ELISA failed until days 90–158 after birth. Specific antibodies against BVDV also impaired the detection of viral RNA in leukocytes and blood. Mean RNA levels of the five calves were reduced in sera 2.500-fold and in leukocytes 400-fold, the lowest values were at week three of life. In contrast, levels of measurable viral RNA in biopsy samples remained constant during the observation period.

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1. Introduction

The bovine viral diarrhoea virus (BVDV) causes high economic losses due to reproductive, enteric and respiratory diseases in cattle. Persistently infected (PI) animals are the major source of virus for transmission. PI animals originate in fetal infection within the first four months of gestation before immunocompetence of the foetus develops (Coria and McClurkin, 1978; McClurkin et al., 1984; Moennig and Liess, 1995). Because PI animals shed large amounts of BVDV throughout their lives (Coria and McClurkin, 1978; Houe, 1995) early detection and elimination of these individuals is a key requirement for BVDV eradication in control programs. To date, most common methods for detecting PI calves are RT-PCR assays and antigen capture ELISAs. Virus isolation and immunohistochemistry are laborious and rarely applied (Edmondson et al., 2007; Fulton et al., 2009; Hilbe et al., 2007).

Maternal antibodies in PI calves can interfere with diagnostic testing. This may even lead to false-negative results, a phenomenon known as “colostral diagnostic gap”. BVDV isolation from blood samples of PI calves is inhibited by maternal neutralizing antibodies for several weeks (Brock et al., 1998; Meyling and Jensen, 1988; Palfi et al., 1993; Zimmer et al., 2004). Testing blood leukocytes with NS3-capture ELISA can produce false-negative results in the first months of life of PI calves (Brinkhof et al., 1996;
Zimmer et al., 2004). This colostro diagnostic gap is less pronounced when capture ELISAs for the soluble BVDV-Erns in blood sera are used (Greiser-Wilke et al., 2003; Sandvik, 2005). In contrast, BVDV-specific PCR with blood samples is believed to be a reliable method for testing PI animals also in the presence of colostal antibodies (Gaede et al., 2003; Rossmanith et al., 2001).

In PI animals high amounts of BVDV are found in hair glands, hair follicles and in epidermal cells and skin biopsy samples can be used for BVDV diagnostics with immuno-histochemistry (Thür et al., 1996a,b). For antigen-capture ELISAs with skin samples, the detection of secreted soluble BVDV-Erns (Rumenapf et al., 1993) seems to be most suitable (Cornish et al., 2005; Huchzermeier et al., 2004). Indeed, when Holmquist tested dried ear biopsy samples collected from eleven PI calves that had received maternal antibodies with an Erns ELISA, all samples reacted positive for Erns while serum samples collected at the same time were negative (Kühne et al., 2005). In terms of virus detection PCR is described as an appropriate method for BVDV screening using fresh or desiccated ear samples (Fux, 2007; Kennedy et al., 2006). By now millions of ear biopsy samples collected by animal tagging were tested with different methods in several European BVDV control programs (AU, CH, I, D) (Oettl et al., 2010; Presi et al., 2011; Tavella et al., 2008). Importantly, a significant proportion of false-negative diagnosis of BVDV-PI calves was noticed. However, the reasons for diagnostic failure are commonly unknown, and, to our knowledge, there are no data available from follow-up studies monitoring the development of virus loads in skin biopsy samples.

In this study we tested the duration and the degree of the impact of colostro-derivèd antibodies on the BVDV detection in PI calves. We clearly demonstrate various reasons for false-negative results when BVDV loads are measured in blood samples or biopsy samples with NS3- and Erns-antigen capture ELISAs, or in virus infected cells with flow cytometry or with real-time RT-PCR. Highly consistent virus detection required the use of dried ear biopsy samples and analysis of viral RNA with an appropriate RT-PCR.

2. Materials and methods

2.1. Persistently BVDV-infected calves, colostro feeding and sample collection

Five BVDV-naive Brown Swiss heifers in early pregnancy had accidentally contact with a BVDV-1 PI animal on a common pasture. Because of seroconversion we expected an intrauterine infection and the development of PI calves. Heifers were housed in a quarantine unit until they gave birth to five healthy BVDV PI calves (A, B, C, D and E). Births were spontaneous at the expected time. The calves were clinically healthy over the whole observation period. We offered repeatedly re-warmed colostro to the calves of the respective cow ad libitum. The intake volumes in the first 24 h of life were 4.0 (A), 6.3 (B), 9.6 (C), 7.5 (D) and 4.3 (E) litres, respectively. Until 72 h of life the calves had drunk 12.4 (A), 16.7 (B), 24.1 (C), 20.5 (D) and 15.8 (E) litres of colostrum. EDTA blood, serum and a skin biopsy samples were collected immediately after birth. Subsequently blood samples were collected daily, skin biopsy samples the following ten days every third day. From days 10 to 28 days of age all samples were collected twice a week and after that time once weekly. Native and EDTA stabilized blood samples were collected by puncture of the jugular vein. Skin biopsy samples were collected applying a 6-mm biopsy punch lateral at the neck.

2.2. Sample preparation

After blood clotting sera were prepared by centrifugation and stored in aliquots at −80 °C. Leukocyte pellets were obtained from EDTA blood following haemolysis in ice-cold ammonium chloride (8.29 g/L NH₄Cl, 1.0 g/L KHCO₃, 1 mM EDTA). For PCR analysis cells were washed in PBS (centrifugation 5 min, 500 × g) and stored in aliquots of 5 × 10⁶ cells at −80 °C. For BVDV-NS3 detection 50 μl whole blood aliquots were haemolysed in 96-well plates and leukocytes were washed in PBS before immediate immunostaining.

The skin biopsy samples were divided into four aliquots within 1 h after collection. To mimic the storage conditions in ear tag sampling devices, biopsies were sealed in 2-ml vials with hygroscopic molecular sieve (Merck, Darmstadt, Germany, no. 105734). Dried samples were stored at +4 °C until testing.

2.3. Real-time RT-PCR

RNA isolation for real-time RT-PCR was performed with RNasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) for biopsy samples, NucleoSpin Virus Core Kit (Macherey-Nagel, Düren, Germany) for serum and High Pure Viral RNA Kit (Roche, Penzberg, Germany) for leukocytes according to the manufacturers’ instructions, respectively.

Dried skin biopsies were homogenized in 300 μl RLT buffer (part of RNeasy Mini Kit) with a 5-mm steel bead in 2-ml vials using a mixer mill MM300 (Retsch, Haan, Germany, 2.5 min at 25 Hz). Subsequently 500 μl proteinase K solutions (part of RNeasy Mini Kit) were added for 10 min at 55 °C. After centrifugation (500 × g, 2 min), total supernatants were completely used for RNA isolation. The NucleoSpin Kit for RNA extraction from 100 μl sera was processed with a workstation (Hamilton Robotics, 100 μl elution volume), all other extractions were performed manually (50 μl elution volume).

The 5’ untranslated region of the BVDV-RNA was detected using a real-time RT-PCR protocol designed by Hoffmann et al. (2006). For the one-step RT-PCR the QuantiTect™ Probe RT-PCR Kit (Qiagen, Hilden, Germany) was used. Five microliters of RNA template were added to 12.5 μl Master Mix, 5.25 μl RNase-free water, 0.25 μl RT Mix and 2 μl primer-probe-mix. The following temperature profile was chosen: reverse transcription 50 °C for 30 min, reverse transcriptase inactivation and polymerase activation 95 °C for 15 min, 42 cycles of denaturation for 30 s at 95 °C, annealing for 45 s at 57 °C and elongation for 45 s at 68 °C. A Strategene MX3005P was used for fluorescence-measurement (Agilent Technologies, Waldbronn, Germany). A log10 dilution series of in vitro transcribed...
BVDV-RNA was included as a quantity standard (Hoffmann et al., 2005).

2.4. Flow cytometry for NS3 detection in leukocytes

Leukocytes were fixed with paraformaldehyde (1%, 10 min on ice), washed three times with PBS and permeabilized with 0.01% digitonin (Fluka, Buchs, Switzerland, no. 37006) for 5 min at room temperature. Indirect immunofluorescence staining was done with pestivirus NS3-specific monoclonal antibodies WB103/105 (Veterinary Laboratory Agency, Weybridge, UK, Lot MAB 07/18, 1:500, 15 min at room temperature) and goat anti-mouse Alexa 488 conjugate (Invitrogen, Eugene, Oregon, USA, Lot 655435, 1:1000, 10 min at room temperature). The conjugate was cross-adsorbed with BVDV-antibody-free native adult cattle serum. Counterstaining was done with propidium iodide (Sigma P4170, 10⁻⁶ mol in PBS). The measurement of 5,000 leukocytes was performed with a FACScan (Becton Dickinson, Heidelberg, Germany). The rate of immunofluorescence positive gated myelocytic cells was calculated with the CellQuest Software (Becton Dickinson).

2.5. Virus neutralisation test

Sera were tested with a virus neutralisation test (VNT) versus the homologues BVDV strain, isolated from calf B at day 181 of life. The sera were inactivated (30 min, 56 °C) and diluted (log₂; 50 μl) with minimal essential medium (MEM, Sigma, Deisenhofen, Germany) in triplicates in 96-well plates. BVDV antibody positive and negative sera were used as controls. 100 TCID₅₀ of BVDV in 50 μl cell culture medium (MEM with 5% BVDV-naïve cattle serum) were added and the plates were incubated 1 h at 37 °C, before bovine turbinate cells (BT, provided by FLI, Riems, Germany; 2 × 10⁴ cells/well in 100 μl cell culture medium) were added. The virus titre was confirmed by back-titration (log₂, n = 6). The plates were incubated for 4–5 days at 37 °C and 5% CO₂. After incubation, the fixation, permeabilisation and indirect immunofluorescence staining were done as described in Section 2.4. Evaluation was performed using an inverted fluorescence microscope. Titres were calculated as the geometric mean values (n = 3) of the reciprocal last dilution in which no BVDV-NS3 specific immunofluorescence signals were observed.

2.6. Antigen and antibody ELISAs

For Erns detection the Herdcheck BVDV Ag/ Serum Plus ELISA was used (IDEXX Europe BV, Schiphol-Rijk, The Netherlands). In skin samples NS3 antigen and NS3-specific antibodies were detected with PrioCheck BVDV Ag PL plus, in sera NS3-specific antibodies were tested with PrioCheck BVDV Ab (Prionics, Martinsried, Germany). Skin specimens were incubated over night (16–18 h) at +4 °C in “ear notch tissue soaking buffer” (Erns ELISA) or at room temperature in “extraction buffer” (NS3 ELISA) provided with the test kits, respectively. The ELISAs were done according to the manufacturers’ instructions. For quantitative analyses of the sera and the skin sample lysates, 1:4 dilution series with PBS were prepared with every sample for endpoint titration. OD values respectively inhibition values according to the testkit of every dilution were listed in a spread sheet (Microsoft Excel) and visualized as titration curves. The calculated dilutions at the intersection points of the titration curves and the ELISA cut-off line were defined as antigen or antibody endpoint titres, respectively. The endpoint titres were calculated by log/ linear interpolation of the result values of the two dilutions adjacent to transition from above (a) to below (b) the cut off value (c). The calculation formula is $4^{-\frac{(\log_{10}(a)-\log_{10}(c))}{(\log_{10}(a)-\log_{10}(b))}}$ with x as exponent of the highest dilution with positive result.

3. Results

3.1. Virus neutralising and BVDV-NS3 specific antibodies in sera

Before first intake of colostrum we detected low levels of BVDV-NS3 specific antibodies (titres of one up to 26) in sera from all five calves. A low virus neutralising titre of 3 was observed in two calves (A and E), three calves were with negative results. Already one day after colostrum feeding maximum virus neutralising and NS3 antibody titers were achieved in every calf (Fig. 1). Against the homologous BVDV isolate we measured virus neutralising titres of 7200 (A), 14,400 (B), 18,100 (C), 64,000 (D) and 35,900 (E). A rapid decrease of titres was observed over several weeks. In sera of four calves the virus neutralisation titres fell down below the detection limit (titre of 2) on days 65 (A), 41 (B), 40 (C), and 87 (E), respectively. In calf D low virus neutralising activity was measured after the decline (day 74) consistently over 4 weeks (titres of 4−7, Fig. 1a). Maximum endpoint titres of NS3-ELISAs ranged from 1000 (A) to 3500 (E). After a plateau of one to three weeks titres declined with a half-life of about 20 days (Fig. 1b). Last sera with detectable NS3-specific antibodies were collected on days 196 (A), 147 (B), 187 (C), 214 (D), and 213 (E).

3.2. BVDV-NS3 positive blood neutrophils and monocytes

As expected for PI animals, we found large proportions of BVDV-NS3-positive myelocytic leukocytes (43–58%) before intake of colostrum (Fig. 2). Thereafter, however, the number of NS3-positive cells rapidly decreased and reached the detection limit (1%) within the first seven days. Importantly, we failed to reliably identify BVDV-NS3 positive myelocytic leukocytes for a period of four (B and C) to nine weeks (E) defining a relevant Colostral gap for this assay. Thereafter, levels of BVDV-positive cells rapidly increased again reaching a plateau of about 30% of IF-positive cells within one week (Fig. 2).

3.3. BVDV-NS3 antigen and BVDV-NS3-specific antibodies in skin biopsy samples

Next, we monitored for NS3 antigen or NS3-specific antibodies in skin samples (Fig. 3). Already before colostrum intake, the detection of NS3 antigen in skin biopsy samples was unreliable. The NS3 antigen ELISA revealed low titres in two animals (B and D) and biopsy
samples collected from calves A, C and E remained negative. Thus, it was not surprising that after colostrum intake we failed to detect BVDV-NS3 in all biopsies collected until day 97 (B), 103 (C), 150 (E), 154 (A) or 166 (D) after birth, respectively. After this extended time period, NS3 antigen was more readily found, albeit at low titres. The maximum titre was 14, for some biopsies only undiluted lysates gave positive results and in two samples (C day 110, D day 186) NS3 was not detectable at all (Fig. 3).

Before colostrum intake, we identified very low amounts of NS3-specific antibodies (titre 1.5) in the skin biopsy sample of one calf (B) only. In contrast, after colostrum intake, all biopsy samples were positive until day 33 (C), 34 (B), 53 (D), 56 (A) and 66 (calf E), respectively. Thereafter, positive NS3 antibody ELISA results were obtained sporadically (C day 61, D day 67 and 74). After day 74, BVDV-NS3 antibodies remained undetectable in all samples.

3.4. BVDV-Erms in serum and in skin biopsy samples

Contrary to NS3 antigen, BVDV-Erms antigen was readily found with high ELISA titres in precolostral sera ranging from 1740 (A) to 7630 (D) (Fig. 4). After colostrum intake, however, we were unsuccessful to detect Erns in any of the sera until days 5 (C), 7 (B) or 14 (A, D, E) postpartum. Thereafter, titres strongly increased within a three-week period reaching levels comparable to those observed before feeding of colostrum.

Similarly, Erns antigen was easily detected in ear skin biopsy samples collected before colostrum intake (Fig. 4). Precolostral Erns ELISA titres ranged from 68 (A), 622 (B), 803 (E), 943 (C) to 1018 (D). Erns antigen remained detectable in skin samples even after feeding with colostrum, albeit at strongly reduced levels. Samples taken on day two (D) or three (A–C, E) scored low Erns titres of 6 (A), 7 (D), 44 (C), 59 (B) or 75 (E). In the following days the amounts of detectable Erns steadily increased and reached levels similar to initial values at the age of three weeks (Fig. 4).

3.5. BVDV-specific RNA in sera, blood leukocytes and in skin biopsy samples

Finally, we assessed the influence of colostrum antibodies on the detection of viral RNA in sera, blood leukocytes and ear skin biopsy samples (Fig. 5). In serum samples of newborn calves we measured virus loads corresponding to a mean value of 10^{0.1} copies BVDV-specific RNA per μl. After colostrum feeding, RNA loads declined to a mean value of 10^{2.7} copies until day 18 and went up again to 10^{5.9} on day 70. Similarly the mean values of virus load in 5 × 10^7 leukocytes fell from initially 10^{7.3} down to 10^{4.7} being followed by an ascent to 10^{6.9} RNA copies. The overall lowest value of a single serum sample was 10^{2.1} RNA copies and 10^{4.6} RNA copies in 5 × 10^5 leukocytes, respectively. Thus, colostral antibodies clearly impaired the detection of viral RNA in serum and blood leukocytes. The large variability of BVDV virus load in the second month of live in sera and leukocytes (Fig. 5) corresponded to the individually different duration of reduced viremia (Fig. 2). The period of low virus load in blood extended to week four in calves B and C, week seven in A, eight in D and to week nine in calf E.

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In sharp contrast to the analysis of blood samples, the presence of colostrum antibodies had no relevant effect on virus load measurements in skin biopsy samples. In the first ten weeks of life the mean value of RNA copies varied between $10^{6.0}$ and $10^{6.6}$ per $5 \mu l$ template on the 15 individual sampling days. The overall lowest value was $10^{3.7}$ and the highest was $10^{7.0}$.

4. Discussion

The aim of this study was to determine the impact of virus-specific, colostrum-derived antibodies on BVDV detection in PI calves. In this context, we detected a sharp increase of BVDV specific virus neutralising and BVDV-NS3 specific antibodies in all five newborn calves after feeding with colostrum. Colostrum is considered to be the sole source of passively acquired maternal antibodies for calves. Yet, we found in all five newborn calves BVDV-NS3 specific antibodies even before colostrum intake. It is generally assumed, that the uninjured placenta epitheliochorialis of cattle does not allow the transfer of antibodies from the cow to the foetus (Tizard, 2004). Nevertheless, we believe that some amount of maternal antibodies can cross the placenta physiologically. This assumption is in agreement with the frequent detection of IgG in precolostral sera of calves (Chigerwe et al., 2008). A consequence, antigen detection might be masked by maternal antibodies even in absence of colostrum intake.

BVDV-NS3 and Erns antigens are both targets of commercially available antigen capture ELISAs for BVDV diagnostics. In our study we failed to reliably detect BVDV-NS3 antigen by ELISA in skin biopsy samples collected before colostrum intake. In contrast, monitoring for BVDV-Erns resulted in successful antigen recognition in samples from each PI animal (Figs. 3 and 4). Possible reasons for this discrepancy could include instability/stability of the antigens in the sample, sensitivity of the test and the specific nature of the target antigen in the specimen. Indeed, in previous investigations we observed that storage of fresh skin samples at temperatures higher than room temperature caused reduced amounts of detectable antigen. This effect was more tremendous for NS3 than for Erns (Fux, 2007). In our study samples were dried

![Image](image_url)

**Fig. 3.** BVDV-NS3 antigen and BVDV-NS3 specific antibodies in skin biopsies. Tests were done with commercialized antigen capture and antibody blocking ELISAs.

![Image](image_url)

**Fig. 4.** BVDV-Erns in skin biopsies and in sera. Tests were done with a commercialized antigen capture ELISA.

![Image](image_url)

**Fig. 5.** BVDV-RNA in skin biopsies, leukocytes ($5 \times 10^5$) and sera ($5 \mu l$) ($N =$ five calves). Viral RNA was extracted in $50 \mu l$ (skin samples or $5 \times 10^6$ leukocytes) or $100 \mu l$ (100 $\mu l$ serum) elution buffer, respectively. In each case $5 \mu l$ were used as template for qPCR.
immediately after collection and were not exposed to critical temperatures. Thus, a deterioration of the samples can likely be excluded. However, we revealed a higher sensitivity of the Erns-ELISA compared to the NS3-ELISA as clearly shown by the results. Erns titres in biopsy samples ranged from 360 to 1750 after a short period of titre reduction due to colostral antibodies. On the contrary, NS3 titres in biopsy samples reached low levels from 1.5 to 7.8 after a long time period with detectable NS3 antibodies in blood (see Figs. 1, 3 and 4 b). These data are concordant with results obtained with different commercially available Erns and NS3 ELISAs and using samples from PI animals at an age well after the possible influence of colostrum antibodies (Fux, 2007). A special comment has to be made with regard to the combined testing of NS3 antigen and antibody with skin specimens. Thereby, a recommended practise is that a negative result for NS3 antigen has to be validated by a negative NS3 antibody result with the same sample to define a “not persistently infected calf”. On the basis of this diagnostic procedure and since we detected neither antigen nor antibodies in most biopsies from month two to five, our five calves would likely be misdiagnosed during the first half year of life. Moreover, in the first month after colostrum feeding, all biopsy samples contained detectable NS3 antibodies, which make it impossible to obtain a proper validation of the NS3 antigen test.

In blood the fractions of BVDV-NS3 positive monocytes and neutrophils declined continuously to the detection limit in the first week of life (Fig. 2). We consider the disappearance of infected leukocytes to be a consequence of in vivo virus neutralisation and the turnover of leukocytes in blood. Importantly, the time window of strong in vivo neutralisation with VN titres in sera above 100 could be determined to be four (calves B, C) to ten (E) weeks in our test group of five PI calves (Fig. 1a). Moreover, quantitative data from PCR using leukocytes or sera as samples confirmed the impact of colostrum on virus loads due to in vivo neutralisation by BVDV-specific antibodies. We measured a thousand fold reduction of BVDV-RNA over the first two weeks of life with lowest virus load being observed in the third week of life.

In contrast, BVDV-Erns in sera was undetectable immediately after colostrum intake but for a short period only. The titres rapidly increased again reaching a maximum plateau in all individuals within two weeks. An obvious explanation for the different detection kinetics of virus load and Erns in blood is the typical property of the envelope associated Erns to be readily shed as soluble protein from infected cells (Fetzet al., 2005; Rumenapf et al., 1993). Therefore, it can be assumed that Erns in blood may originate from miscellaneous infected tissues and the colostral gap for Erns detection disappears upon clearance of Erns-specific maternal antibodies. It still remains to be determined whether Erns antibodies are transferred at lower concentrations initially or whether the formation of Erns-specific immunocomplexes results in a more rapid depletion of these antibodies as compared to neutralising antibodies or NS3-specific antibodies. We also consistently observed a strong blocking effect of colostral antibodies for the detection of Erns in skin biopsy samples. Compared to the first biopsy collected before colostrum intake, the second biopsy sample from day two or three revealed at least 10-fold lower amounts of antigen. The strongest decline of titre was seen with biopsy samples of calf D dropping from an initial Erns titre of 1018 to 6.7 on day two. The Erns blocking effect can be explained by the presence of antibodies from blood and lymph in the specimen. As shown in Fig. 3, BVDV-specific antibodies are found in biopsy specimens. In extracts from biopsy samples NS3 antibody titres were about 100-fold lower as compared to those in sera. The variability in antibody titre is probably caused by different amounts of blood in the biopsy samples. In the field, PI calves are expected to individually vary with regard to the infecting BVDV strain, the time point of fetal infection, and the amount of transferred colostral antibodies. In addition, the quality of the diagnostic specimens might be lower than in our study. Thus, our data may not be fully representative for field conditions. In our opinion it can be expected, that the screening of newborn calves using ear biopsies and Erns-capture-ELISA will lead to false-negative classification in a small proportion of calves. Indeed, we have obtained first strong evidence for such misdiagnosis in eight of 233 young PI calves assessed in a field investigation (unpublished data). In addition, Erns ELISA monitoring of biopsy samples from newborn PI calves reported a similar range of 2.7% false-negative classifications in Tyrol in Austria recently (Oettl et al., 2010).

The amount of BVDV-RNA in skin biopsies is not influenced by in vivo virus neutralisation, in contrast to results from blood samples (Fig. 5). Immunohistological investigations show that the BVDV is widely distributed in the skin of PI animals (Thür et al., 1996b), and detection of BVDV antigen in skin samples of PI animals with immunohistochemical methods is possible also during the colostral period (Braun et al., 1999; Grooms and Keilen, 2002). BVDV is located in the stratum basale and the stratum spinosum of the epidermis as well as in the isthmus and the infundibulum of hair follicles (Njaa et al., 2000; Thür et al., 1996a). The elimination of skin cells, infected with non-cytopathic BVDV, would largely depend on a specific cellular immunity. However BVDV-specific T cells are not available in immunotolerant PI animals. In contrast to skin cells, virus infected blood leukocytes are likely cleared by the natural turnover: Thus, infected cells disappear continuously during the first week after colostrum intake and neutralising antibodies are expected to prevent the infection of newly recruited myeloid cells. From these results we hypothesize that resting stem cells are not infected by BVDV. After the decline of the virus neutralising activity below a critical value, leukocytes can become infected again.

A direct comparison of the sensitivity of PCR for routine diagnostic procedures using serum versus purified blood leukocytes cannot be deduced from our results (Fig. 5). The amount of about 8 μl blood for 5 μl serum or about 600 μl blood for 5 x 106 purified leukocytes is very different.

In conclusion, our data confirm that colostral-derived antibodies may strongly influence the detection of BVDV in PI calves by various methods. Importantly, when using dried ear biopsy samples as diagnostic specimens the
application of BVDV-NS3 ELISAs must be considered generally insufficient in the presence of maternal antigens. The impact on BVDV detection conducted with Erns ELISAs is less extensive, yet, we expect false-negative results at a small rate. The most reliable detection of BVDV PI animals required the solubilisation of high quality RNA from dried biopsy samples and PCR analysis with evidence for 100% sensitivity even in the presence of maternal antibodies. These data are of utmost importance for a reliable distinction of non-PI and PI calves in ongoing BVDV eradication programs.

Conflict of interest

None.

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References


