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Transplacental and oral transmission of wild-type bluetongue virus serotype 8 in cattle after experimental infection

Anoek Backx*, René Heutink, Eugene van Rooij, Piet van Rijn

Department of Virology, Central Veterinary Institute of Wageningen University and Research, Houtribweg 39, 8221 RA, Lelystad, the Netherlands

*Corresponding author: anoek.backx@wur.nl, +31 320 238 898, fax:+31 320 238 668

Correspondence address: Anoek Backx
Department of Virology
Central Veterinary Institute of Wageningen UR
P.O.Box 65
8200 AB, Lelystad
The Netherlands
Anoek.Backx@wur.nl
Abstract

Potential vertical transmission of wild-type bluetongue virus serotype 8 (BTV-8) in cattle was explored in this experiment. We demonstrated transplacental transmission of wild-type BTV-8 in one calf and oral infection with BTV-8 in another calf. Following the experimental BTV-8 infection of seven out of fifteen multi-parous cows eight months in gestation, each newborn calf was tested prior to colostrum intake for transplacental transmission of BTV by RRT-PCR. If transplacental transmission was not established the calves were fed colostrum from infected dams or colostrum from non-infected dams spiked with BTV-8 containing blood. One calf from an infected dam was born RRT-PCR positive and BTV-specific antibody (Abs) negative, BTV was isolated from its blood. It was born with clinical signs resembling bluetongue and lived for two days. Its post-mortem tissue suspensions were RRT-PCR positive. Of the seven calves fed colostrum from infected dams, none became infected. Of the six calves fed colostrum from non-infected dams spiked with infected blood, one calf became PCR-positive at day 8 post partum (dpp), seroconverted 27 days later, and remained RRT-PCR and Abs positive for the duration of the experiment (i.e. 70 dpp). This work demonstrates that transplacental transmission in late gestation and oral infection of the neonate with wild-type BTV-8 is possible in cattle under experimental conditions.
1. Introduction

Bluetongue virus (BTV) (family *Reoviridae*, genus *Orbivirus*) is an arthropod-borne double-stranded RNA virus with 24 serotypes that can cause the non-contagious infectious disease bluetongue (BT) in ruminants. BT is a World Organization for Animal Health (OIE) reportable disease and is of considerable socioeconomic concern and of major importance in the international trade of animals and animal products. Since 1998 southern and central Europe have seen incursions of five different serotypes of BTV (1, 2, 4, 9 and 16) (Mellor and Wittmann, 2002; Saegerman et al., 2008; Schwartz-Cornil, 2008). A sixth serotype, BTV-8, was first identified in northern Europe in August 2006 (Elbers et al., 2008b; Saegerman et al., 2008). Its recrudescence in 2007 in the Netherlands, Germany, Belgium, Luxembourg and northern France, and its subsequent emergence in the United Kingdom, Denmark, Switzerland and the Czech Republic, has raised the question how BTV-8 survives between vector seasons.

Some of the hypotheses on this overwintering ability of BTV are prolonged or persistent infection in vertebrate hosts, the persistence of the virus within surviving adult vectors during the winter, and transovarial transmission through the vector (Purse et al., 2005; Sellers and Mellor, 1993; Takamatsu et al., 2003). The transmission of the virus between its ruminant hosts is almost exclusively by the bites of *Culicoides* midges. Of the indigenous European *Culicoides* species, principally *C. dewulfi*, the Obsoletus complex, and recently found *C. chiopterus*, can be infected with BTV-8 (Dijkstra et al., 2008; Mehlhorn et al., 2007; Meiswinkel et al., 2007) and might turn out to be competent vectors. The distinct feature that BTV-8 survives the winter by passing from pregnant infected ruminants to their offspring while *in utero*, is raised following observations in the Netherlands and Northern Ireland in March 2008 that PCR-positive ruminant offspring were born to dams that were infected with bluetongue virus the previous year (Menzies et al., 2008; van Wuijckhuise, 2008), and by a Belgian study of cattle and sheep in the field (De Clercq et al., 2008). Additionally the reports
of aborted and newborn calves from BTV-8 infected dams with severe developmental defects of the brain, i.e. hydranencephaly, proves natural vertical transmission of the European strain of BTV-8 (De Clercq et al., 2008; Vercauteren, 2008). Before this, it was generally believed that wild-type BTV lacks the capability for transplacental transmission, whereas cell adapted BTV can (Gibbs et al., 1979; Kirkland and Hawkes, 2004). Concerning other transmission routes of BTV, oral transmission, including oral transmission, was suggested recently for BTV-8 in ruminants (Menzies et al., 2008) and was shown only once by a repeated experiment for other serotypes in sheep (Jochim et al., 1965; Luedke and Jochim, 1968), while it was demonstrated in carnivores (Alexander et al., 1994; Jauniaux et al., 2008).

BTV-8 present in northern Europe is unusual in its transmission by novel vectors and the ability to cause disease and mortality in cattle (Elbers et al., 2008a; Schwartz-Cornil, 2008), also other transmission routes (De Clercq et al., 2008; Menzies et al., 2008; Vercauteren, 2008; Wouda et al., 2008) seem to be part of the new characteristics, but experimental evidence applying serotype 8 in ruminants to support the field observations is missing.

The objective of this work was to explore the potential transplacental and oral transmission of wild-type BTV-8 after experimental infection in cattle. Therefore the calves born from infected multi-parous dams were tested immediately after birth for transplacental BTV-8 infection and subsequently allocated to a feed-group to test for possible oral BTV-8 infection.

2. Materials and Methods

2.1. Animals

2.1.1. Pregnant multi-parous cows
This study was conducted by permission of the Animal Ethics Committee of the Central Veterinary Institute of Wageningen UR. Following pre-screening on clinical health, the absence of BTV by RRT-PCR and of BTV-specific antibodies (Abs), and on being late in gestation with the expected calving date around mid-February 2007, sixteen pregnant multiparous Holstein-Friesian cows were commercially sourced from different Dutch holdings. They were housed together in a tie-stall a week before inoculation. During this week they were gradually adjusted from rough silage to grass pellets, observed for clinical signs, and screened for BTV Abs and virus in serum and blood and for Bovine Virus Diarrhoea Virus (BVDV) shedders by antigen ELISA. One cow calved before the onset of the experiment and was left out. Seven of the remaining fifteen cows were infected with BTV-8 as described below. The other eight cows served as non-infected dams. Nets, fully covering the udder, were attached to each cow to prevent newborn calves from drinking colostrum directly.

2.1.2. Calves

Newborn calves from the cows were housed in single calf boxes positioned separately in the housing of the dams. Blood and serum samples were taken from the calves straight after birth and before colostrum intake. The BTV status from these samples determined the allocation to a particular feed-group. After three days being fed with colostrum from the allocated feed-group the calves were gradually adjusted from colostrum to a commercial milk-replacer and milk pellets.

2.2. Virus origin

2.2.1 BTV-8 inoculate to infect the cows

The virus used to infect cows in this experiment was BTV-8 from field infected pregnant Dutch Holstein-Friesian heifers in 2006, estimated as at least day 40 to 50 post
infection (Thiry et al., 2006), passed once as full blood through BTV-8 negative Dutch Holstein-Friesian heifers. EDTA blood from the viremic heifers at days 7 and 21 post infection (dpi) was stored at -70°C, thawed before use, and pooled to obtain a sufficient quantity to inoculate the cows for this experiment.

2.2.2. BTV-8 inoculate to spike colostrum from non-infected dams

The virus used to spike colostrum from non-infected Dutch Holstein-Friesian cows was obtained by withdrawal of 500 ml EDTA blood from each of the seven BTV-8 infected multi-parous cows at 8 dpi, i.e. in the acute phase of infection before the detection of BTV-specific Abs. This blood was pooled, stored at -70°C and thawed before use. Per calf 100 ml was divided over the colostrum portions of the first three days, i.e. three liter colostrum per day with one to two volume percent of full blood.

2.3. BTV Tests

2.3.1 BTV RRT-PCR

EDTA blood was tested for the presence of BTV RNA by an in-house developed real-time reverse transcriptase PCR (RRT-PCR) detecting all 24 serotypes of BTV. Briefly, viral dsRNA from 200 μl diluted (1:1 with PBS) EDTA blood was isolated using the Total Nucleic Acid Isolation Kit (Roche Diagnostics Nederland b.v., Almere, Netherlands) in the isolation robot MagNa Pure (Roche Diagnostics Nederland b.v., Almere, Netherlands) according to manufacturer’s instructions. Isolated dsRNA samples were tested using primers (Eurogentec Nederland b.v., Maastricht, Netherlands),

the forward primer is 5’-AGTGTCGCTGCCATGCTATC-3’

and the reverse primer is 5’-GCGTACGATGCGAATGCA-3’,
and a Taqman probe 5'-6FAM-CGAACCTTTGGATCAGCCCGGA-XTMR-PH (Tib MolBiol, Berlin, Germany) targeting segment 10 of the BTV genome. RRT-PCR was performed by use of the LightCycler RNA Master Hybridization Probes kit (Kit, Roche Diagnostics Nederland b.v., Almere, Netherlands) with a LightCycler 2.0 (Roche Diagnostics Nederland b.v., Almere, Netherlands). Template RNA (5 μl) was added to a reaction mixture containing 0.25 μM of the forward and reverse primer, 0.25 μM probe, 2.75 mM MnCl₂, 7.5 μl Kit and 0.2 μl RNAsin (RNAsin, 40U/μl, Promega Benelux b.v., Leiden, Netherlands) in a final volume of 20 μl. Thermocycling conditions of the RRT-PCR were: 20sec 98°C, 20min 61°C, 30sec 95°C, (1sec 95°C, 10sec 61°C, 15sec 72°C)x45 followed by 30sec 40°C and storage at 4°C. Amplification was monitored real time by OD₅₃₀/OD₆₄₀ using software version 4.05 (Roche Diagnostics Nederland b.v., Almere, Netherlands). Three positive controls were included containing different virus dilutions of BTV1 grown on BHK21 cells in DMEM with 5% fetal bovine serum and diluted in the same growth medium. The weak positive control, containing the highest virus dilution, must result in a visible curve therewith determining the cut-off. DNAse free phosphate-buffered saline (PBS) solution was used as negative control. A run was successful when all negative controls were negative, and positive controls were positive. Results of test samples were considered positive if the software generated a crossing point (cp) value and a sigmoid curve with a signal (OD₅₃₀/OD₆₄₀) at least partly above the cut-off value. Results were considered doubtful in case of a sigmoid-shaped curve completely below the cut-off value, or of all other not interpretable curves.

2.3.2. BTV ELISA

Sera were tested for BTV-specific Abs in a commercial ELISA (ID Screen® Bluetongue Competition ELISA kit, ID Vet, Montpellier, France) according to manufacturer’s instructions.
2.3.3. BTV Virus isolation

Virus isolation (VI) was performed on embryonated chicken eggs (ECE) according to the OIE Manual (OIE, 2007). After one passage on ECE, homogenates were prepared and the supernatant was tested in the BTV RRT-PCR.

2.4. BVDV Tests

2.4.1. BVDV Antigen ELISA

To detect possible BVDV shedders, EDTA blood was tested in a commercial BVDV antigen ELISA (IDEXX Europe b.v., Hoofddorp, Netherlands) according to manufacturer’s instructions.

2.4.2. BVDV Antibody ELISA

Sera were tested to detect BVDV-specific Abs in a commercial ELISA (Prionics Lelystad, Lelystad, Netherlands) according to manufacturer’s instructions.

2.4.3. BVDV Virus isolation

Virus isolation (VI) was performed, following the method described by Smith et al. (Smith et al., 1988) modified by using an anti-BVDV hyper immune serum (HIS) of pigs instead of monoclonals and the use of DAKOPATTS conjugate labelled with anti-pig-HRPO (Wensvoort et al., 1986). Serum samples were tested instead of the ideally used buffy coat of blood samples. Because BVDV VI was performed in retrospect haemolysis of the samples due to frozen storage did not allow for testing of the buffy coat.

2.5. Study design
2.5.1. Dams: BTV infection

Seven multi-parous Holstein-Friesian cows, eight months pregnant, were inoculated with BTV-8, 20 ml i.v. in the v.jugularis and 20 ml s.c. on the right side of the neck, i.e. the infected dams (I). The other eight pregnant cows were not inoculated, i.e. the non-infected dams (NI). EDTA blood and serum samples were collected daily in the week following the day of inoculation (d0), every other day in the second week, and once a week thereafter. These samples were tested using the RRT-PCR for the detection of BTV RNA and the ELISA for the detection of BTV-specific Abs. Body temperature was recorded daily, with fever defined above 39.5°C. The animals were observed and physically examined every day and any clinical signs were scored using a clinical reaction index (CRI) modified from that of Huismans and others (Huismans et al., 1987). The administration of analgesic and antimicrobial treatment was allowed. The cows were let to calve spontaneously. After calving the cows were milked at least two days to collect colostrum and than euthanized by intravenous injection of a combination of 200 mg/ml embutramide, 50 mg/ml mebezoniumjodide and 5 mg/ml tetracaine hydrochloride (T61; Intervet Nederland b.v., Boxmeer, Netherlands).

2.5.2. Calves: Transplacental BTV transmission

Immediately after birth the calves were tested for possible transplacental transmission of BTV. The EDTA blood sample and serum sample drawn straight after birth and before the first ingestion of colostrum were tested for BTV by RRT-PCR and ELISA, respectively. According to the outcome of the tests, each calf was allocated to the appropriate feed-group in order to be able to study BTV infection by ingestion of colostrum. When the calves tested positive for BTV, they were allocated to a feed-group with colostrum from non-infected dams (col-NI).
2.5.3. Calves: oral BTV infection

Cows were let to calve naturally and spontaneously, randomizing the order of birth from infected or non-infected dams. Inducing the calving process in any way was not acceptable, also concerning the fact that the precise conception dates were unknown. The calves born uninfected were allocated to a feed-group, i.e. colostrum from infected dams (col-I) or colostrum from non-infected dams spiked with blood from infected dams (col-NI+bld-I). Each calf was fed 3 litre of the appointed colostrum per day for at least three days. The colostrum uptake was assured by visual inspection of the amount taken in by the calves together with the turbidimetric determination of the concentration of total Immunoglobulin G ([IgG]) in calf sera at 3 dpp by the Animal Health Services in The Netherlands.

EDTA blood and serum samples were collected daily in the week of birth (d0), every other day in the second week, and once a week thereafter. These samples were tested using the RRT-PCR for the detection of BTV RNA and the ELISA for the detection of BTV-specific Abs. Body temperature was recorded daily, with fever defined above 39,5°C. The animals were observed and physically examined every day and any clinical signs were recorded. The administration of analgesic and antimicrobial treatment was allowed. If the clinical signs in the animals became so severe that their welfare was unacceptably compromised, they were euthanized. At the end of the experiment the calves were euthanized by intravenous injection of a combination of 200 mg/ml embutramide, 50 mg/ml mebezoniumjodide and 5 mg/ml tetracaine hydrochloride (T61; Intervet Nederland b.v., Boxmeer, Netherlands).

3. Results

3.1. Dams: BTV infection
All of the seven BTV-8 infected dams were RRT-PCR positive at three dpi and BTV specific Abs positive at 12 dpi (Fig. 1). Five of the infected dams developed clinical signs. These signs started with the loss of appetite, anorexia, depression, salivation, nasal discharge and conjunctivitis. Three of these dams had very high CRI scores (Tab I). They developed severe lesions of face and feet, i.e. hyperaemic mucosa of nose and mouth, lesion of oral and/or nasal mucosa, painful feet, oedema of the legs, severe coronitis, recumbency due to laminitis or coronitis (Fig. 2). The clinical signs are conform the clinical signs caused by BTV-8 reported in cattle from the field in northern Europe (Elbers et al., 2008a; Elbers et al., 2008b).

The eight non-infected dams remained RRT-PCR and Abs negative, no clinical signs were observed.

All dams pre-tested negative for BVDV antigen in the antigen ELISA. Before inoculation with BTV-8, seven of the sixteen dams had BVDV-specific antibodies, i.e. 965, 966, 969, 970, 973, 974 and 975. Of the BTV-8 inoculated dams that did not have BVDV antibodies before inoculation, 967, 977 and 978 sero-converted to BVDV-specific antibodies between 7 and 15 dpi (partus at 11 dpi), 7 and 22 dpi (partus 23 dpi), and 9 and 12 dpi (partus 33 dpi), respectively. Non-cytopathogenic BVDV was isolated at 7 dpi for dam 967 and 977 and at 12 dpi for dam 968 (partus at 22 dpi). The non-infected dams did not seroconvert to BVDV and no BVDV was isolated.

Six of the seven infected dams gave birth to six calves, the calf of the other infected dam, dam 977, died in utero and during the caesarean section the moment of death was antedated at least two days. Seven non-infected dams gave birth to eight calves. Non-infected dam 963 was diagnosed with a torsio uteri, and during the caesarean the calf was also found dead in utero.
3.2. Calves: Transplacental BTV transmission

Calf 987 from infected dam 978 was born with a hyperaemic nose and hyperaemic conjunctivae of the right eye (Fig. 3). The pre-colostrum tests resulted in a RRT-PCR Cp value of 21.6, in comparison, the average Cp value of the maximum positive controls in all the PCR runs was 27. The ELISA was negative for BTV-specific Abs. This calf died two days after it was born (see Table II). Post-mortem tissue suspensions of spleen, liver, kidney, lung, nose, mesenteric lymphnode, heart, brain were all RRT-PCR positive. Bluetongue virus was isolated from blood of the day of birth of this calf.

The calves from the other infected dams were RRT-PCR and Abs negative at birth. From the calf of dam 977 that died in utero, spleen and liver tissue was tested by RRT-PCR with a negative outcome. The calves from the non-infected dams were born with negative BTV pre-colostrum test results.

All calves, from infected and non-infected dams, were born without BVDV-specific antibodies. BVDV virus isolation was performed on the serum samples and on spleen and liver tissue suspensions from calf 987 and the calf from dam 977, which were all negative.

3.3. Calves: oral BTV infection

Allocation to a feed-group of each calf was according to its BTV status at birth, i.e. transplacental transmission, and by the BTV infection status of its respective dam (see Table II). Calf 987 was the only calf infected with BTV at birth and hence fed with col-NI. The other calves were given colostrum from infected dams (col-I) (N=7) or colostrum from non-infected dams spiked with BTV-8 (col-NI+bldI) (N=6). Maternal antibody intake per calf was detected by measuring the total [IgG] in serum at 3 dpp, excluding calf 986 (dam-I col-I) that died at 1 dpp. Eleven calves took in maternal antibodies by colostrum as indicated by [IgG] in serum at 3 dpp. Only marginal uptake of maternal antibodies was detected for calf 989,
although it did drink the total offered volume of colostrum. BTV-specific Abs were detected from day 1 in all calves fed col-I, except for earlier mentioned calf 989 (only at 12 dpp) and calf 983. The marginally low intake of maternal antibodies by colostrum intake of calf 989 might explain the fact that BTV-specific antibodies could only be detected at one day. Calf 983 remained negative for BTV Abs as it was fed colostrum from milkings of the day of birth from its own dam 967, who gave birth at 11 dpi, one day before she became Abs positive (from 12 dpi). All calves with BTV-specific Abs except for 988, dropped below detection level before the end of the experiment. All six calves who were fed with BTV-specific Abs free colostrum, i.e. col-NI+bld-I, remained negative for BTV-specific Abs, except for calf 979. This calf 979 (dam-NI, col-NI+bld-I) was RRT-PCR positive from 8 dpp, with a Cp of 24.6 (peak at 21 dpp of Cp 21.9), and BTV-specific Abs were detected from day 35 pp. This calf remained BTV and BTV-specific Abs positive until the end of the experiment, i.e. 70 dpp.

All calves except for 989 had BVDV-specific antibodies at the day they left the experiment.

4. Discussion

This work provides indications to support recent field observations that BTV-8 present in northern Europe can be transplacentally transmitted to offspring of infected dams. Moreover, this work provides evidence that oral transmission of wild-type BTV-8 might also occur. In short: Experimentally transplacental transmission with BTV-8 was detected in one calf (987) born from a dam infected late in gestation, and oral infection was detected in one calf (979) from a non-infected dam fed with colostrum from a non-infected dam spiked with blood from the infected dams.
Transplacental transmission of bluetongue virus has been described before for cattle (De la Concha-Bermejillo et al., 1993; Luedke et al., 1977a, b; MacLachlan et al., 2000; Waldvogel et al., 1992), sheep (Flanagan and Johnson, 1995; Richardson et al., 1985) and dogs (Brown et al., 1996; Osburn, 1994; Wilbur et al., 1994), but only with cell-attenuated virus strains. Experiments with wild-type bluetongue virus did not result in vertically transmitted bluetongue virus (Acree et al., 1991; Hubbert et al., 1972; Parsonson et al., 1994), except for a study with BTV11 in two elk cows in which a criticized method for virus detection has been used (Stott et al., 1982). Regarding the effect of the moment of infection of the dam in gestation, only infections early in gestation have been studied with cell attenuated virus. Late-term infection has only been studied by directly infecting bovine fetuses through the uterine wall (Jochim, 1974; Waldvogel et al., 1992).

The finding in this study that transplacental transmission of wild-type BTV-8 after experimental infection of a pregnant cow late in gestation is possible, provides support for the recent observations in the Netherlands and Northern Ireland (Menzies et al., 2008) and in Belgium (De Clercq et al., 2008) that PCR-positive ruminant offspring are being born to dams that were infected with BTV-8, and the increase in reports of aborted and newborn calves from BTV-8 infected dams with severe developmental defects of the brain, i.e. hydranencephaly (Vercauteren, 2008; Wouda et al., 2008). The importance of vertical and oral transmission in general, and in particular for the overwintering and spread of BTV remains unclear, but definitely depends on the significance of the natural BTV-spread in a respective season, area or country. At the same time it raises some immediate questions, as to whether BTV-8 present in northern Europe stems from an attenuated strain, and if there are conditions to facilitate the incidental BTV crossing over the placenta, like for instance interaction with co-infections, and whether the recent findings could explain a possible overwintering mechanism.
Regarding the potential role played by co-infections, interaction with BVDV as a known placenta crosser (Hewicker-Trautwein et al., 1994) could be a target for further discussion and research. After all, dam 978 of calf 987 did seroconvert to BVDV after inoculation with BTV-8, but so did other BTV infected dams without delivering a BTV positive calf. The only difference between these dams that seroconverted to BVDV after BTV-8 infection was that the dam 978 of calf 987 seroconverted to BVDV 11 days or more prior to delivery and the others just before or around the partus date. She also delivered her calf 10 days later than the others. Therefore she could have been infected 10 days earlier in her pregnancy than the other dams, whereby both BTV and BVDV could have had more time to cause vascular damage and hence interact to infect the calf in utero. Although no BVDV-specific antibodies could be detected in the calves at birth and no BVDV could be isolated from liver and spleen suspensions from calf 987, BVDV might have helped BTV to cross the barrier.

Oral transmission of BTV-8 was detected in one calf out of thirteen calves fed with potentially infecting colostrum. This calf, 979, born from a non-infected dam was fed with spiked colostrum. As oral transmission of BTV has not been described for ruminants before, except once for domestic sheep with BTV10 (Jochim et al., 1965; Luedke and Jochim, 1968) and for wild carnivores by eating infected prey (Alexander et al., 1994), this outcome might reveal a different incidental route of virus transmission for BTV-8.

The underlying hypothesis for oral BTV transmission was that virus could leak into the colostrum and that by drinking this infected colostrum calves could become infected. BTV is known to be associated with endothelial cells, to interact with the cell membrane of erythrocytes and therewith to be able to evade neutralizing antibodies, and to replicate in dividing lymphocytes (MacLachlan, 1994). Blood cells leaking into colostrum or milk in the mammary gland could therefore transport the virus. To study whether this happens under
natural conditions, seven calves born BTV negative were fed with colostrum from infected
dams. Furthermore, to mimic virus-infected colostrum without BTV-specific antibodies as
might be the case when a dam gets infected very late in gestation, the colostrum from non-
infected dams was spiked with BTV-8 infected blood without BTV-specific antibodies. This
was fed to six calves born BTV negative.

Regarding evidence of virus transmission by colostrum or milk, there are not many
studies to be found. Maedi Visna virus (MVV) can be transmitted to lambs by feeding
colostrum of infected ewes (Alvarez et al., 2005; Preziuso et al., 2004). Foot-and-Mouth
disease virus (FMDV) is shed in milk, which can than be spread (Tomasula and Konstance,
2004). For calf bovine leukemia virus (BLV), colostral transmission of BLV to neonatal calves
is possible if the dam is co-infected with bovine immunodeficiency virus (BIV) and BLV
(Meas et al., 2002). However in the case of dams being infected with only BLV, colostral
antibodies are shown to be protective and thus to prevent colostral BLV transmission (Nagy et
al., 2007). In comparison, the colostrum from infected dams fed to seven calves, except 983,
contained BTV-specific Abs. These antibodies might have neutralized the potential presence
of BTV virus in the colostrum, thereby protecting the calves against BTV infection. Equally,
the absence of BTV-specific Abs in the spiked colostrum fed to six calves might have
provided an opportunity for the virus to infect a calf by ingestion. Still, to reach more
conclusive results regarding the potential oral transmission of BTV in the presence of BTV-
specific antibodies further study is needed. Ideally this is done by spiking colostrums that
contain different titers of BTV-specific Abs with different amounts of BTV.

5. Conclusion

In conclusion, transplacental transmission in late gestation of wild-type BTV-8 was
demonstrated by this study in one calf after experimental infection of the dam, as was oral
BTV-8 infection of the neonate by infected colostrum. Our findings support the possibility of alternative transmission routes for BTV-8 that may play a part in explaining its overwintering between vector seasons. To elucidate the unique characteristics of this BTV-8 strain further research is required, for instance to identify its ability to cross the placenta at a molecular level. Furthermore it may lead to clues about the origin of the BTV-8 strain present in northern Europe.

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Tables

Table I. Clinical scores* of seven infected mothers: multi-parous Holstein-Friesian cows infected experimentally with wild-type BTV-8 approximately one month before calving

<table>
<thead>
<tr>
<th>Mother</th>
<th>Days scored</th>
<th>Fever</th>
<th>Anorexia</th>
<th>Face</th>
<th>Feet</th>
<th>Total score</th>
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<td>D</td>
<td>D/DS*4</td>
<td>D</td>
<td>D/DS*4</td>
<td>0-4</td>
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<td>1</td>
<td>0.2</td>
<td>8</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
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</tbody>
</table>

* Clinical scores were calculated for the clinical signs developed between day one after inoculation and the moment of euthanasia as follows. Fever: the number of days (D) with a temperature>39.5°C divided by the total number of days scored (DS) after inoculation times 4, rounded up to one decimal; Anorexia: calculated like Fever score for days with less or no appetite; Lesion score: Face (rhinitis, conjunctivitis, salivation, hyperaemia, ulcers of mucosa, facial oedema), Feet (lameness, coronitis, recumbency due to inflamed feet) each scored from 0-4 if present with 4 being the most severe. The range of the total score is 0 to 16.
Table II. Wild-type BTV-8 (non)-infected dams and transplacental transmission and oral infection with BTV-8 of their respective calves.

<table>
<thead>
<tr>
<th>Dams Group</th>
<th>ID a</th>
<th>BTV infection † b</th>
<th>Calves</th>
<th>BTV transplacental</th>
<th>Colostral</th>
<th>BTV infection † b</th>
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<td></td>
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<td>PCR ELISA (+ in dpi)</td>
<td>ID</td>
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<td>Feedgroup [IgG]</td>
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<td>(dpi dams)</td>
<td>(at 0 dpp)</td>
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</tr>
</tbody>
</table>

a ID = identification number  
b † = day of euthanization/death  
c dpi = days post infection  
d dpp = days post partum  
e The concentration (in gram per liter) of total Immunoglobulin G was tested in serum from the calves three days post partum, as an indicator for the intake of antibodies from colostrum.  
f feedgroups: col-I: colostrum from infected dam; col-NI+bld-I: colostrum from non-infected dam spiked with blood from infected dam; col-NI: colostrum from non-infected dam  
g ND = not done, this calf lived for only 1 day.  
h caesarian section, calf found dead antedated at least 2 days  
i torsio uteri, calf found dead with caesarian  
j This calf was born before the onset of the experiment and therefore excluded  
k the numbers between brackets refer to clinical signs of the infected dams in table I and Fig.1  
l Dam 971 gave birth to a twin, calves 979 and 980.
Figure captions

Figure 1. Onset and duration of positive test results for bluetongue virus (BTV) PRC, ELISA, fever (T>39.5°C), and recorded clinical signs of bluetongue in days post inoculation (dpi) with wild-type bluetongue serotype 8 for seven multi-parous Dutch Holstein-Friesian cows infected at eight months gestation. Note that the scale is discontinous after 7 dpi. The numbers above the bars mark the duration for the individual infected mothers. † indicates the death of the numbered animal.

Figure 2. Severe clinical signs of bluetongue in wild-type BTV-8 infected Dutch Holstein-Friesian dams.

a) Mucous nasal discharge at 19 dpi

b) Severe general illness, apathy, anorexia, at 20 dpi

c) Severe coronitis at 21 dpi (blue discoloration of coronary band)

Figure 3. Hyperaemic nose of transplacentally BTV-8 infected calf.
Figures:

![Graph showing BTV PCR, BTV ELISA, T > 39.5°C, and Clin. signs with days post inoculation (dpi)]

Figure 1.

![Image of a cow with clinical signs](image_url)

Figure 2a.
Figure 2b

Figure 2c
### Tables

**Table I. Clinical scores* of seven infected mothers: multi-parous Holstein-Friesian cows infected experimentally with wild-type BTV-8 approximately one month before calving**

<table>
<thead>
<tr>
<th>Mother</th>
<th>Days scored</th>
<th>Fever</th>
<th>Anorexia</th>
<th>Face</th>
<th>Feet</th>
<th>Total score</th>
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<td>0.4</td>
<td>12</td>
<td>1.5</td>
<td>7.8</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Dams</th>
<th>Calves</th>
<th>Colostral</th>
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<tbody>
<tr>
<td>Group</td>
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