Effects of Border disease virus (genotype 3) naturally transmitted by persistently infected sheep to pregnant heifers and their progeny

R. Krametter-Froetscher[1], N. Mason[1], J. Roetzel[1], V. Benetka[2], Z. Bago[3], K. Moestl[2], W. Baumgartner[1]

[1]Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria
[2]Clinical Virology, Department for Pathobiology, University of Veterinary Medicine Vienna, Austria
[3]Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Moedling, Austria

ABSTRACT: Eight heifers pregnant between days 47 and 73 were kept together with nine healthy persistently Border disease virus (BDV)-infected sheep allowing natural contact comparable to field conditions. All heifers seroconverted between days 23 and 38 after exposure. Besides a mild increase in body temperature in four heifers, no clinical signs of infection were observed, but 5 animals aborted between days 54 and day 202 after exposure. BDV was detected in the aborted fetuses of four heifers and in the placenta of the fifth (the only material available). Foetal mummification was seen in three foetuses, aborted between days 113 and 116 of gestation, with a crown rump length (CRL) of between 11 and 12 cm. The associated placentas showed dystrophic calcification. The foetus aborted on day 267 of gestation had a CRL of 70 cm and a body mass of 16 kg. The brain in this case was normal in terms of gross morphology, but histologically slight lymphocytic meningeal and perivascular infiltration, slight demyelination in the cerebellar white matter and slight focal acute liquefactive necrosis in the thalamus were seen. Three heifers delivered clinically healthy calves. Two healthy calves were pestivirus negative, of these one was serologically positive and one negative (precolostral). The third calf was pestivirus positive and antibody negative at birth, suggesting immunotolerance and persistent infection as has been described for BVDV. But surprisingly when retested at an age of seven months the calf had seroconverted and was pestivirus negative. Post-mortem examination of the heifers and the calves born alive revealed no abnormalities, pestivirus specific RNA was not detected in any of the examined organ samples of the eight heifers and the three calves. In this study, BDV-type 3 infection had a disastrous impact on fertility of pestivirus naïve heifers. The observed abortion rate exceeded 50% and dramatically underlines the need for a pestivirus control program which includes BDV and small ruminants.

Keywords: Border disease virus; abortion; sheep; BVDV eradication; cattle

Abbreviations
BDV = Border disease virus; **BVDV** = Bovine viral diarrhea virus; **RT-PCR** = reverse transcriptase polymerase chain reaction; RNA = ribonucleic acid; ELISA = enzyme-linked immunosorbent assay

Border disease virus (BDV) is an important sheep pathogen causing significant losses in sheep farming worldwide (Nettleton et al., 1998). Together with Bovine viral diarrhoea virus (BVDV) and Classical swine fever virus (CSFV) it belongs to the genus *Pestivirus* within the family *Flaviviridae*

(Fauquet et al., 2005). BVDV, the causative agent of Bovine viral diarrhoea and of Mucosal disease (MD), is an economically important pathogen of cattle (Nettleton and Entrican, 1995). Genetic typing of several pestivirus isolates of sheep and goats originating from different countries allocated them to at least three different genotypes (Vilcek et al., 1997; Pratelli et al., 2001; Giangaspero and Harasawa, 2004 a,b; Stalder et al., 2004). Sequence and phylogenetic analyses further demonstrated that interspecies transmission of closely related ruminant pestiviruses is possible and occurs in the field. Krametter-Froetscher et al. (2008a) described BVDV-1h in a goat originating from a mixed flock of goats and cows in Vorarlberg, a Federal state of Austria. In cases of direct contact to sheep, BDV infection of cattle is probably not unusual in the field. Several field infections have been reported in the very recent past. The transmission of BDV from persistently infected sheep to calves was documented by Krametter-Froetscher et al. (2008c) in a study imitating field conditions. Hornberg et al. (2009) detected BDV-3 in a cow in Austria, originating from Tyrol, a Federal state of Western Austria, which borders the provinces Salzburg and Vorarlberg. In the case described by Hornberg et al. (2009) sheep were suspected as the source for BDV infection. A clinically healthy calf from Salzburg persistently infected with BDV-3 was documented by Krametter-Froetscher et al. (2009). In Vorarlberg and Salzburg, Krametter-Froetscher et al. (2007a, 2008b) described BDV-3 in sheep with a high similarity at the nucleotide level to the isolate documented by Hornberg et al. (2009) and Krametter-Froetscher et al. (2009). Further cases have been described in England and Wales (Cranwell et al., 2007; Strong et al., 2010).

Clinically, transient pestivirus infections in ruminants usually cause no or only mild symptoms such as inappetence, moderate fever, oculo-nasal discharge, mild diarrhoea and decreased milk production (Nettleton and Entrican, 1995). However, the pathogenicity of BDV and BVDV isolates can vary and depends on the virulence of the strain and the immunity of the host. Also in immunocompetent ruminants severe clinical cases caused by pestivirus infections, particularly those involving the type 2 viruses, have been reported (Nettleton and Entrican, 1995; Carman et al., 1998; Nettleton et al., 1998; Liebler-Tenerio et al., 2003).

The main clinical signs of BDV infection in sheep and of BVDV in cows are seen in pregnant animals: embryonic/foetal death and absorption or abortion, mummification, congenital defects, stillbirth and the birth of weak or persistently infected offspring (Brownlie et al., 2000; Otter et al., 2009). Within this wide range of symptoms, the effect of the infection depends particularly on the gestational stage at which the infection occurs. If cows are infected between approximately 30 and 125 days of gestation, BVDV may cross the placenta, infect the foetus and induce persistent infection (Brock et al., 2005). The persistently infected animals shed virus throughout their lifetime and therefore they are the main BVDV reservoirs (Nettleton and Entrican, 1995; Nettleton et al., 1998; Brock et al., 2005).

Epidemiological studies have shown that Pestiviruses in cattle, sheep and goats are distributed worldwide. Serological studies among the cattle population in Europe have shown individual prevalences between 17% and 84% and herd prevalences of up to 100% (Houe, 2005). Houe (2005) described a prevalence rate of persistently BVDV infected cattle in Europe of up to 2.1% and a prevalence rate of persistently infected herds of up to 53%. Serological studies carried out among the sheep populations in several European regions revealed individual prevalences of between 5% and 70% and herd prevalences of between 8% and 90% (Loken et al., 1991; Schaller et al., 2000; Tegtmeier et al., 2000; Graham et al., 2001; Krametter-Froetscher et al., 2007b). Investigations among goats have shown that the seroprevalence rate was generally lower than in cattle and sheep (Loken, 2000; Tegtmeier et al., 2000; Krametter-Froetscher et al., 2006). However, marked regional differences in the seroprevalence among cattle, sheep and goats were seen. These variations were attributed to differences between regions, management practices, the density of the populations of animals and the levels of movement (Loken et al., 1991; Graham et al., 2001; Houe, 2005; Krametter-Froetscher et al., 2006, 2007b). In Austria, in sheep pastured during summertime on communal alpine meadows an individual seroprevalence of 89% and a herd prevalence of 44% were described (Krametter-Froetscher et al., 2007b). Additionally, in Austrian sheep and goat populations with close contact to cattle, the individual and herd seroprevalences were higher than in small ruminants without cattle contact (Krametter-Froetscher et al., 2006; 2007b).

At the beginning of the 1990s, when the important role of persistently infected cattle in transmitting BVDV was established and the financial

damage caused by the disease was recognized, several European states initiated national and regional control programs for BVDV (Sandvik, 2004; Houe et al., 2006). In 1997, several Austrian regions started voluntary BVDV programs, especially among breeding cattle (Obritzhauser et al., 2005; Rossmanith et al., 2005; Schoepf et al., 2005). Schoepf et al. (2005) described an effective reduction of persistently infected cattle from 1.22% to 0.37% due to a voluntary BVDV eradication program in the Federal state Tyrol, where communal Alpine pasturing of cattle, sheep and goats is a century-old farming practice. The success of voluntary BVDV eradication programs in Austria was also described by Rossmanith et al. (2005) among cattle on common grasslands in Lower Austria.

In August of 2004, a national compulsory BVDV control program started in the cattle population in the entire country of Austria. The purpose of this program is the identification and eradication of all cattle persistently infected with BVDV. If this program proves successful, the Austrian cattle population should be free of BVDV and also free of antibodies to BVDV in the nearer future. In Austria, a pestivirus epidemiology among sheep exists, independent from cattle (Krametter-Froetscher et al., 2007a,b; 2008b). Krametter-Froetscher et al. (2008c) showed that sheep persistently infected with BDV may induce transient BDV infection in calves.

Very little information is available about the impact of BDV infection on fertility and pregnancy of cattle and if it can cause similar consequences as infection with BVDV (low fertility, abortion, persistent infection). The aim of this study was to investigate the effects of BDV-genotype 3 infection from naturally persistently infected sheep on susceptible pregnant cattle and their progeny. Moreover, the results of the study carried out should finally establish whether sheep persistently infected with BDV represent a risk factor for the success of the Austrian BVDV control program as suspected in previous studies (Hornberg et al., 2009; Krametter-Froetscher et al., 2008b,c).

MATERIAL AND METHODS

Animals. The experiment included eight clinically healthy pregnant Simmental heifers, purchased from herds with no history of BVDV. The heifers were all tested and shown to be negative for pestiviruses insteated of BVDV and antibodies against the virus. At a gestation stage of between 47 and 73 days (Table 1) the heifers were exposed to nine healthy naturally persistently Border disease virus (BDV-3)-infected sheep.

The study was carried out at a quarantine stable, where the sheep and the heifers had close contact in the stable and in the yard. Additionally, the sheep had a separate pen in the stable to which the heifers had no access. All animals were handled in accordance with ethical standards, and experimental protocols were approved by the Ethics Committee of the Veterinary University of Vienna and the Austrian Ministry for Health.

Clinical examination, monitoring of pregnancy and sample collection from exposed heifers. Routine clinical procedures (Baumgartner, 2005a) were carried out every third day until seroconversion was recognized. Afterwards, clinical examination was performed weekly. Behaviour and appetite were monitored daily by the keepers.

All heifers were examined every third day for gross evidence of abortion and for pregnancy at four week intervals via rectal exploration and transrectal ultrasonography (Medison SonoVet 2000, Germany). Whenever a dead foetus was detected during ultrasonography abortion was induced using prostaglandine (Estrumate®).

Blood samples were collected by jugular vein puncture as well as nasal and conjunctival swabs from each heifer every third day after exposure to the sheep until pestivirus antibodies were detected. The same sampling procedure was performed on the day of abortion and of parturition, respectively. Milk samples were collected on the day of parturition (Day 1), on Day 2 and on Day 3.

At the end of the experiment, all heifers were euthanized and examined in a routine manner for gross pathological and histological changes. RT-PCR was performed on selected organ samples of all heifers (spleen, lung, liver, thymus, kidneys, thyroid gland, brain, udder and ovary).

Serological, virological and haematological examinations. Pestivirus antibodies were detected using two commercially available enzyme-linked immunosorbent assay (ELISA) kits (BDV-Ab and BVDV-Ab; Svanova Biotech AB, Uppsala, Sweden) according to the manufacturer's instructions.

RT-PCR for pestivirus specific RNA was performed on blood samples, nasal and conjunctival swabs, milk samples, faeces, abortion materials as well as on organ samples (thyroid gland, thymus,

lung, spleen, liver, kidneys, brain, uterus, ovaries and udder) of euthanised animals. Previous to the RNA extraction, faeces were resuspended in DEPC-treated water and centrifuged, whereas nasal and conjunctival swabs were stored in DEPC-H2O. Leucocyte buffy coat, milk and tissue samples were lysed by proteinase K digestion (10 vol% Proteinase K in Buffer ATL, Qiagen, Hilden, Germany) at 56°C. RNA extraction was done using a commercially available viral RNA Kit (QIAamp viral RNA Kit, Qiagen, Hilden, Germany) following the manufacturer's instructions. For the detection of pestivirus RNA the primers 324 and 326 amplifying a conserved domain of the 5'-end of the viral genome (288 bp) were used (Vilcek et al., 1994). Virus genotyping of pestivirus positive samples was carried out using RT nested PCR amplifying a 452 bp segment of the Npro region as described by Vilcek et al. (1997) and Becher et al. (1999).

A complete white blood cell count was performed using ADVIA® 120 (Bayer Diagnostic, Germany). Haematological results were interpreted according to the guidelines of Baumgartner (2005b).

Post-mortem examination of aborted materials and serology of attendant heifers. Aborted materials (foetuses, placentas) were collected and submitted to routine necropsy and histological examination as well as RT-PCR and sequence analysis.

The following bacteriological examinations were performed to rule out the aetiological involvement of pathogenic bacteria. Chlamydophila were excluded by means of fluorescence antibody test (IMAGEN™-Chlamydia, Dako, Ltd., England) according to the manufacturer's instructions. Serum samples collected from the heifers immediately after abortion were tested for specific antibodies against *Brucella abortus*, Bovine herpesvirus type 1, *Leptospira* ssp., *Chlamydophila psittaci, Coxiella burnetii, Neospora caninum, Toxoplasma gondii.* Pathomorphological investigation, bacteriology and serology described above were performed following the standard operating protocols of the Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Moedling.

Clinical examination and sample collection of live born calves. Routine clinical examination was performed on the day of birth, one and three days later, and afterwards at weekly intervals. Immediately after birth and before calves had any opportunity to ingest colostrum, blood samples were taken for molecular, serological and haematological analyses, using procedures previously mentioned. In addition, nasal and conjunctival swabs, and faeces were taken at birth and analysed by RT-PCR. One day and four days after birth the same approach was followed. In case of a positive result (detection of BDV RNA), the samples described above were collected and investigated again on Day 7 after birth. Calves were maintained alive for several weeks depending on molecular and serological results.

After all examinations were finished, calves were euthanized and necropsied. Histological and molecular analyses were performed on selected organ samples (thyroid gland, thymus, lung, spleen, liver, kidneys, brain, ovaries or testes).

Immediately before euthanasia serum samples were collected again for serological studies.

RESULTS

Clinical, serological, virological, haematological and pathomorphological findings in exposed heifers

The heifers were bright, alert and eating well throughout the total period of the study. The body temperature did not exceed 39°C with values equal or below 38.8°C in most cases. Within the ten day period prior to seroconversion a mild increase in body temperature was observed three times in heifer 1 (once 39°C; twice 38.9°C) and once in heifer 2 (39°C), 4 (39°C) and 8 (38.9°C).

All heifers serconverted between days 23 and 38 post viral exposure (Table 1) and remained seropositive throughout the experiment.

All blood and milk samples, nasal and conjunctival swabs and organ samples tested negative for pestivirus specific RNA (Table 1).

White blood cell counts were within normal ranges in heifers 1, 2, 3, 4, 7, 8 (normal range 6200 to 9500 cells/µl; Baumgartner, 2005b). In heifer 5 a mild increase in total white blood cells was seen 5 days and 7 days before seroconversion (9860 cells/µl and 9990 cells/µl, respectively). In heifer 6 the highest value was 13 470 cells/µl (one day before seroconversion) and the lowest value 6660 cells/µl (one day after seroconversion). Only heifer 7 showed once (16 days before seroconversion) a mild decrease in lymphocytes (2993 cells/µl).

No relevant gross pathological or histological changes were found by post-mortem examination of the heifers.

Abortion and neonatal death rates

Heifers 1, 3, 6, 7 and 8 aborted between days 113 and 267 of gestation corresponding to days 54 and 202 after first exposure to the PI sheep (Table 1). In heifers 3 and 8 abortion was induced by prostaglandine after foetal death.

Post-mortem examination of aborted materials and serology of attendant heifers

Foetal mummification was seen in three foetuses aborted between day 113 and day 116 of gestation with a crown rump length (CRL) of 11 to 12 cm (one foetus of heifer 1, twins of heifer 8). As far as the poor condition of the foetuses allowed the pathological study, no gross abnormalities or microscopic lesions compatible with pestiviral infection were observed.

Pathomorphological examination of the placentas of heifers 1 and 8 revealed moderate dystrophic calcification. Additionally, in the placenta of heifer 1, leucocytostasis of the blood vessels and moderate neutrophilic and lymphohistiocytic infiltration of the stroma were found. In heifer 6 only the placenta was available. Cotyledons were grey-white discoloured and histologically showed oedema and a slight vascular and stromal infiltration with lymphocytes and plasma cells.

Heifer 3 aborted a female foetus with a CRL of 21 cm on day 132 of gestation. Gross findings revealed, aside from a severe mummification (particularly in the brain), no specific alterations. Histologically, the lung showed a moderate interstitial infiltration with lymphocytes and atelectasis. In the liver, beside active haematopoiesis, a moderate leucocytostasis of the liver sinusoids was seen. In heifer 3 the placenta was not available.

The female foetus aborted on day 267 of gestation by heifer 7 had a CRL of 70 cm and a body mass of 16 kg. The coat was longer than normal. Gross findings revealed that the lung was ventilated but dystelectatic. The mesenterium showed a moderate oedema. All other organs, including the brain were normal. Histologically, in the heart multifocal myocardial necrosis with partial dystrophic calcification were seen. The dystelectatic lung showed evidence of aspiration of amniotic fluid and meconium, the liver mild periportal lymphocytic infiltrates, while glycogen storage was observed in the hepatocytes and examination of the kidneys revealed moderate pigment nephrosis. In the brain a slight lymphocytic meningeal and perivascular infiltration, slight demyelination in the cerebellar white matter and slight focal acute liquefactive necrosis in the thalamus were seen.

The placental stroma showed a slight oedema and lymphoplasmocytic infiltration.

Table 1. Clinical and laboratory findings in heifers and pathological findings in their progeny

Heifer	Clinical and laboratory findings in heifers				Observations in progeny, pathological findings
	day of gestation at first exposure	day of seroconversion after viral exposure	viral RNA in blood, swabs, milk	abortion (days after first exposure)	
1	62	32	negative	yes (54)	lesions in placenta; foetus mummified
2	47	23	negative	no	none
3	62	32	negative	yes (70)	no placenta available; foetus mummified
4	73	32	negative	no	none
5	55	38	negative	no	none
6	59	23	negative	yes (57)	lesions in placenta. no foetus available
7	65	32	negative	yes (202)	lesions in placenta; foetus with long coat; lesions in brain and heart
8	54	26	negative	yes (59)	lesions in placenta; 2 foetuses mummified

Table 2. Detection of antibodies in blood and RNA in blood, nasal, conjunctival swabs and faeces and white blood cell findings in calves 2, 4, 5

	Calf 3			Calf 4			Calf 5			
	Day 1*	Day 2	Day 5	Day 1*	Day 2	Day 5	Day 1*	Day 2	Day 5	Day 8
Serum antibodies[1]	–	–	+	+	+	+	–	+	+	+
RNA/blood[2]	–	–	–	–	–	–	+	+	+	+
RNA/swabs[2]	–	–	–	–	–	–	–	–	–	–
RNA/faeces[2]	–	–	–	–	–	–	+	–	–	–
Leukocytes (µl)	9 690	7 520	6 620	6 290	7 750	7 480	22 750	10 680	9 410	
Neutrocytes (µl)	6 288	3 699	2 648	3 899	2 797	2 928	19 337	5 980	5 363	
Lymphocytes (µl)	3 110	3 376	3 905	2 113	3 634	3 976	1 137	4 165	3 387	

*day of birth before colostrum intake; [1]antibody positive = +; antibody negative = –; [2]RNA positive = +; RNA negative = –

Bacteriological investigations performed on aborted materials and serological investigations carried out on serum samples of the heifers showed negative results.

Pestivirus specific RNA was found in organ pools of all examined foetuses and in the placenta of heifer 6. The RNA of all positive samples was amplified and typed as BDV-3 (highest identity of 97% to Acc. Nr. AY895009, Swiss BDV-3 strain CH-BD2).

Clinical examination, serology, virology and post-mortem examination from calves born alive

Heifers 2 (calf 2), 4 (calf 4) and 5 (calf 5) showed a normal pregnancy and a normal parturition (Table 1). Clinical examination of the three newborn calves (one male, two females) revealed no clinical abnormalities.

Results of serological, virological and haematological analyses of calves 2, 4, 5 between birth (Day 1), Day 2 and Day 5 are listed in Table 2. The pestivirus-specific sample found in calf 5 was typed as BDV-3.

The calves 2 and 4 were euthanized at an age of four and six weeks, respectively. At this age no antibodies to pestiviruses were detected in calf 2. The serum sample of calf 4 was still antibody positive in both ELISA's.

Calf 5, positive for BDV-3 RNA and seronegative at birth, was euthanized at an age of seven months. At this age pestivirus specific RNA was not found in blood, nasal and conjunctival swabs. Antibodies were detected in the BVDV- and BDV-ELISA. Positive ELISA results were confirmed by virus neutralization tests (VNT's) as previously described (Krametter-Froetscher et al., 2005; 2007b). The results of the VNT's revealed a titre of 1 : 17 400 against the BDV-strain 137/4 and a titre of 1 : 4 300 against the BVDV strain NADL.

In the organs examined after death pestivirus specific RNA was not detected in any of the calves. Pathomorpological investigations revealed no abnormalities in the calves described.

DISCUSSION

In this study we describe for the first time the consequences of a Border disease virus infection transmitted from naturally and persistently infected sheep to susceptible pregnant heifers and their progeny. Typical clinical abnormalities found in cattle infected with highly virulent BVDV strains (Bolin et al., 1985; Liebler-Tenerio et al., 2003) were not seen in the heifers described here. Collins et al. (2009) described an increase in the temperature of calves infected intranasally with BVDV with the peak around Day 8 or 9 and leucopenia between Days 3 and 14 post-challenge. In the study described here, a possible association between the mild increases of the body temperature within the ten day period before seroconversion in four heifers is quite certainly not an effect of the BDV-3 infection. Dubovi (1999) has postulated that pestivirus infection in ruminants occurs around 10 days before seroconversion. Stockstad and Loken (2002) documented no clinical signs of illness in heifers infected experimentally with BVDV-1. Also, heifers infected intramuscularly with BDV showed

no clinical signs of a transient pestivirus infection (Gibbons et al., 1974). Altogether, the BDV strain described in this study seems to have a low virulence for immunocompetent cattle. In agreement with the findings of Stockstad and Loken (2002) all heifers had developed serum antibodies 5 weeks after exposure. In none of the blood samples was viral RNA detectable, a result that possibly can be explained by the fact that transient viraemia in these cases was short and weak.

In any case, the outcome of the infection of the heifers in respect to their progeny was serious: an abortion rate of more than 50% was observed. Gibbons et al. (1974) described foetal loss of 90% in heifers between 117 and 264 days of gestation inoculated intramuscularly with BDV when 50 days pregnant. Abortion as a consequence of BVDV infection occurs frequently in early stages of gestation (less than 125 days of gestation), although abortions in the late phase of gestation have also been described (Liebler-Tenerio et al., 2003). In the face of these facts it seems that the Border disease virus originating from naturally infected sheep as described here has a high analogy with several BVDV strains in the development of the disease in the infected foetuses. The negative effects of BVDV on reproduction in pregnant cows found by Stockstad and Loken (2002) were moderate compared to the results on reproduction described here. Stockstad and Loken (2002) infected 22 heifers in early pregnancy (74–81 days) and reproductive failures such as abortion or stillborn calves were not registered.

In cattle, acute infection with BVDV in the first trimester of gestation possibly leads to a persistent infection of the foetus. Persistent infection results in embryonic death or in the birth of a viable BVDV-positive and antibody-negative calf (Brock et al., 2005). The calf of heifer 2 (pregnant 47 days on the day of exposure to the sheep infected with BDV-3) was at the time of birth BDV negative and precolostral antibodies to BDV were not detected. A probable explanation is that the immune system of the dam eliminated the viremia before the virus passed the placenta and infected the foetus.

Calf 5 was positive for BDV-3 RNA precolostrally and on the following days, but seronegative. According to previous studies (Brock et al., 2005) these results indicate a persistent infection and immune tolerance. However, the calf was retested for a further study at an age of seven months and pestivirus specific RNA was no longer detected; instead a high antibody titre to BDV was found. These findings lead to the conclusion that calf 5 was transiently infected with BDV-3, seroconverted and eliminated the virus. BVDV persistence in acutely infected and antibody positive animals has been reported in reproductive tissues and white blood cells (Grooms et al., 1998; Givens et al., 2003; Collins et al., 2009). Antibodies to the virus in calf 5 were not detected at birth and therefore a possible long term persistence of the virus following *in utero* infection of the immunocompetent foetus is relatively unlikely. It is also possible that a prolonged residence of the virus in the dam with an infection of the foetus at the period of parturition occurred.

However, further investigations are required to elucidate the observed consequences of the BDV-3 infection in calves 2 and 5.

The fact that contact with sheep naturally and persistently infected with BDV leads to seroconversion in heifers as already previously described in calves by Krametter-Froetscher et al. (2008a) and to abortion, is of prime importance for the BVDV control program in Austria. The success of the BVDV program implemented in Austria in 2004 is characterised by a broad decrease of the number of antibody carriers against BVDV and cattle positive to BVDV (Obritzhauser et al., 2005; Rossmanith et al., 2005; Schoepf et al., 2005). The study described here definitively clarifies that especially in regions where sheep and cattle share the same pastures or on farms harbouring sheep and cattle, the success of the Austrian BVDV program is fatally compromised in the face of an existing BDV epidemiology in sheep independent from cattle (Krametter-Froetscher et al., 2007b, 2008b). The introduction of BDV in cattle herds free of BVDV and free of antibodies to BVDV will lead to economic losses due to reproductive failure in Austrian cattle herds and expensive follow up examinations.

Acknowledgements

This work was supported by grants from the Austrian Ministry for Health. We thank Dr. Wodak, Dr. Vanek, Mr. Bauer, Mrs. Steiner, Mrs. Holzer and Mr. Knotzer and his team from the Austrian Agency for Health and Food Safety for their collaboration.

REFERENCES

Baumgartner W (2005a): Untersuchungsgang beim Wiederkäuer, 47–192. In: Baumgartner W (ed.): Klinische Pro-

pädeutik der inneren Krankheiten und Hautkrankheiten der Haus- und Heimtiere. Parey, Stuttgart. 381 pp.

Baumgartner W (2005b): Beurteilung des weißen Blutbildes, 216–217. In: Baumgartner W (ed.): Klinische Propädeutik der inneren Krankheiten und Hautkrankheiten der Haus- und Heimtiere. Parey, Stuttgart. 381 pp.

Becher P, Orlich M, Kosmidou A, Koenig M, Baroth M, Thiel HJ (1999): Genetic diversity of pestiviruses: identification of novel groups and implications for classification. Virology, 262, 64–71.

Bolin SR, McClurkin AW, Coria MF (1985): Effects of bovine viral diarrhea virus on the percentages and absolute numbers of circulating B and T lymphocytes in cattle. American Journal Veterinary Research, 46, 884–886.

Brock KV, Grooms DL, Givens MD (2005): Reproductive disease and persistent infections, 145–156. In: Goyal M, Ridpath JF (ed.): Bovine Viral Diarrhea Virus; Diagnosis, Management and Control. Blackwell, Oxford. 261 pp.

Brownlie J, Thompson I, Curwen A (2000): Bovine virus diarrhoea virus – strategic decisions for diagnosis and control. Practice, 4, 176–187.

Carman S, Van Dreumel T, Ridpath J, Hazlett M, Alves D, Dubovi E, Tremblay R, Bolin S, Godkin A, Anderson N (1998): Severe acute bovine viral diarrhea in Ontario, 1993–1995. Journal Veterinary Diagnostic Investigations, 10, 27–35.

Collins ME, Heany J, Thomas CJ, Brownlie J (2009): Infectivity of pestivirus following persistence of acut infection. Veterinary Microbiology, 138, 289–296.

Cranwell MP, Otter A, Errington J, Hogg RA, Wakeley P, Sandvik T (2007): Detection of Boder disease virus in cattle. Veterinary Record, 11, 211–212.

Dubovi EJ (1999): Bovine viral diarrhea virus and Border Disease virus (Flaviviridae). In: Granoff A, Webster RG (eds.): Encyclopedia of Virology. 2nd ed. Academic Press, London. 173–180.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005): Virus Taxonomy, Classification and Nomenclature of Viruses. Eighth Report of the International Committee on Taxonomy of Viruses. Academic Press, London. 1162 pp.

Giangaspero M, Harasawa R (2004a): Ovine pestiviruses: their taxonomic status revealed by palindromic nucleotide substitutions. Veterinary Microbiology, 70, 33–39.

Giangaspero M, Harasawa R (2004b): Genetic variety of Bovine viral diarrhea virus 2 strains isolated from sheep. Journal Veterinary Medicinal Science, 66, 323–326.

Gibbons DF, Winkler CE, Shaw IG, Terlecki S, Richardson C, Done JT (1974): Pathogenicity of the Border Disease agent for the bovine foetus. British Veterinary Journal, 130, 357–360.

Givens MD, Heath AM, Brock KV, Broderson BW, Carson RL, Stringfellow DA (2003): Detection of bovine viral diarrhea virus in semen obtained after inoculation of seronegative postpubertal bulls. American Journal of Veterinary Research, 64, 428–434.

Graham DA, Calvert V, German A, Mccullough SJ (2001): Pestiviral infections in sheep and pigs in Northern Ireland. Veterinary Record, 148, 69–72.

Grooms DL, Brock KV, Ward LA (1998): Detection of bovine viral diarrhea virus in the ovaries of cattle acutely infected with bovine viral diarrhea virus. Journal of Veterinary Diagnostic Investigations 10, 125–129.

Hornberg A, Revilla-Fernandez S, Vogl C, Vilcek S, Matt M, Fink M, Koefer J, Schoepf K (2009): Genetic diversity of pestivirus isolates in cattle from Western Austria. Veterinary Microbiology, 135, 205–213.

Houe H (2005): Risk Assessment, 38–46. In: Goyal M, Ridpath JF (eds.): Bovine Viral Diarrhea Virus; Diagnosis, Management and Control. Blackwell, Oxford. 261 pp.

Houe H, Lindberg A, Moennig V (2006): Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. Journal Veterinary Diagnostic Investigations, 18, 427–436.

Krametter-Froetscher R, Loitsch A, Moestl K, Baumgartner W (2005): Seroprävalenz von Border Disease und Boviner Virusdiarrhoe bei Schafen und Ziegen in ausgewählten Regionen Österreichs. Wiener Tierärztliche Wochenschrift, 92, 238–244.

Krametter-Froetscher R, Loitsch A, Kohler H, Schleiner A, Schiefer P, Moestl K, Golja F, Baumgartner W (2006): Prevalence of antibodies to Pestiviruses in goats in Austria. Journal Veterinary Medicine B, 53, 48–50.

Krametter-Froetscher R, Kohler H, Benetka V, Moestl K, Golja F, Vilcek S, Baumgartner W (2007a): Influence of communal Alpine pasturing on the spread of Pestiviruses among sheep and goats in Austria: first identification of Border Disease Virus in Austria. Zoonoses and Public Health, 54, 209–213.

Krametter-Froetscher R, Loitsch A, Kohler H, Schleiner A, Schiefer P, Moestl K, Golja F, Baumgartner W (2007b): Serological survey for antibodies against pestiviruses among sheep in Austria. Veterinary Record, 160, 726–730.

Krametter-Froetscher R, Benetka V, Duenser M, Bago Z, Theiner A, Preyler B, Moestl K, Vilcek S, Baumgartner W (2008a): First descriptive study of a pestivirus infection in an Austrian goat. Veterinary Record, 163, 192–194.

Krametter-Froetscher R, Schmitz C, Benetka V, Bago Z, Moestl K, Vanek E, Baumgartner W (2008b): First de-

scriptive study of an outbreak of Border disease in a sheep flock in Austria – a high risk factor for Bovine viral diarrhea virus free cattle herds: a case report. Veterinarni Medicina, 53, 625–628.

Krametter-Froetscher R, Benetka V, Moestl K, Baumgartner W. (2008c): Transmission of Border Disease Virus from sheep to calves – a possible risk factor for the Austrian BVD eradication programme in cattle? Wiener Tierärztliche Monatsschrift/Veterinary Medicine Austria, 95, 200–203.

Krametter-Froetscher R, Benetka V, Rasser K, Tockner F, Moesslacher G, Moestl K, Baumgartner W (2009): BVDV control program in Austria – is a monitoring of the BDV status in sheep in Austria necessary? Veterinarni Medicina, 54, 517–524.

Liebler-Tenerio E, Ridpath JF, Neill JD (2003): Lesions and tissue distribution of viral antigen in severe acute versus subclinical acute infection with BVDV-2. Biologicals, 31, 119–122.

Loken T (2000): Border disease in goats. In: Tempesta M (ed.): Recent Advances in Goats Diseases. International Veterinary Information Service (www.ivis.org).

Loken T, Krogsrud J, Larsen IL (1991): Pestivirus infections in Norway. Serological investigations in cattle, sheep and pigs. Acta Veterinaria Scandinavica, 32, 27–34.

Nettleton PF, Entrican G (1995): Ruminant Pestiviruses. British Veterinary Journal, 151, 615–642.

Nettleton PF, Gilray JA, Russo P, Dlissi E (1998): Border disease of sheep and goats. Veterinary Research, 29, 327–340.

Obritzhauser W, Fuchs K, Koefer J (2005): BVDV infection risk in the course of the voluntary BVDV eradication program in Styria/Austria. Preventive Veterinary Medicine, 72, 127–132.

Otter A, Welchmann DB, Sandvik T, Cranwell A, Hollimann A, Millar MF, Scholes SFE (2009): Congenital tremor and hypomyelination associated with bovine viral diarrhoea virus in 23 British cattle herds. Veterinary Record, 164, 771–778.

Pratelli A, Martella V, Cirone F, Buonavoglia D, Elia G, Tempesta M, Buonavoglia C (2001): Genomic characterization of pestiviruses isolated from lambs and kids in southern Italy. Journal of Virological Methods, 94, 81–85.

Rossmanith W, Janacek R, Wilhelm E (2005): Control of BVDV-infection on common grassland – The key for successful BVDV-eradication in Lower Austria. Preventive Veterinary Medicine, 72, 133–137.

Sandvik T (2004): Progress of control and prevention programs for bovine viral diarrhea virus in Europe. Veterinary Clinics of North America/Food Animal Practice, 20, 151–169.

Schaller P, Vogt H-R, Strasser M, Nettleton PF, Peterhans E, Zanoni R (2000): Seroprävalenz von Maedi-Visna und Border disease in der Schweiz. Schweizer Archiv für Tierheilkunde 142, 145–153.

Schoepf K, Gaede W, Matt M (2005): Erfahrung zur Bekämpfung der Bovinen Virus Diarrhoe (BVD/MD) mit spezieller Berücksichtigung der Verbreitung von BVDV-Genotypen im Bundesland Tirol. Veterinary Medicine Austria/Wiener Tieraerztliche Monatsschrift, 92, 254–258.

Stalder HP, Pfaffen G, Rüfenacht J, Schaller P, Bachofen C, Marti S, Strasser M, Vogt H-R, Peterhans E (2004): Genetic heterogeneity of pestiviruses of ruminants in Switzerland. In: Abstract Proceedings. 2nd European Symposium on BVDV Control, Porto, Portugal, p 16.

Stockstad M, Loken T (2002): Pestivirus in cattle: Experimentally induced persistent infection in calves. Journal Veterinary Medicine B, 49, 494–501.

Strong R, La Rocca SA, Ibata G, Sandvik T (2010): Antigenetic and genetic characterisation of border disease viruses isolated from UK cattle. Veterinary Microbiology, 141, 208–215.

Tegtmeier C, Stryhn H, Uttenthal A, Kjeldsen AM, Krogh Nielsen T (2000): Seroprevalence of Border disease in Danish sheep and goat herds. Acta Veterinaria Scandinavica. 41, 339–344.

Vilcek S, Herring AJ, Nettleton P F, Lowings JP, Paton D J (1994): Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Archives of Virology, 136, 309–323.

Vilcek S, Nettleton PF, Paton DJ, Belak S (1997): Molecular characterization of ovine pestiviruses. Journal of General Virology, 78, 725–735.

Received: 2010–02–10
Accepted after corrections: 2010–04–30

Corresponding Author:

Dr. Reinhild Krametter-Froetscher, University of Veterinary Medicine, Department for Farm Animals and Veterinary Public Health, Clinic for Ruminants, Veterinaerplatz 1, A-1210 Vienna, Austria
Tel. +43 125 077 5232, e-mail: Reinhild.Krametter@vu-wien.ac.at