

BVDV control program in Austria – is a monitoring of the BDV status in sheep in Austria necessary?

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ABSTRACT: In cattle referred to the Clinic of Ruminants at the University of Veterinary Medicine Vienna by local veterinarians from Lower Austria, the number of animals positive for antibodies against pestiviruses decreased from 11.9% in 2004 to 7.4% in 2007. In other Austrian regions the seroprevalence of 17.6% in 2004 dropped to 12.2% in 2007. The seroprevalence rates were considerably higher in older animals than in younger indicating a marked decrease of new infections (8.2% in < 1.5 years old animals, 6.8% in 1.5–4.5 years, 19.8% in 4.5–7.5 years and 33.3% in > 7.5 years). These data nevertheless also demonstrate that new pestivirus infections occur, although at a lower rate. We report the case of a calf persistently infected with Border disease virus-3 (BDV-3) detected in a mixed cattle and sheep farm with the status “BVDV-free”. Earlier investigations have shown that BDV-3 is endemic in Austrian sheep populations and seems to be a potential risk factor for the reintroduction of pestiviruses in BVDV free cattle herds. Serological findings among the investigated sheep population showed in four out of nine sheep samples considerably higher titres to the BDV strain Moredun than to the BVDV strain NADL. Seroconversion against pestiviruses was also detected in contact cattle and the mother of the persistently infected calf. Pestivirus specific RNA was neither detected in the blood samples collected from the patients of the Clinic for Ruminants nor in the sheep or cattle investigated on the farm described.

Keywords: BDV; BVDV; cattle; sheep

The *Pestivirus* genus, within the family *Flaviviridae* comprises four accepted species: Bovine viral diarrhoea virus type 1 (BVDV-1), BVDV-2, Border disease virus (BDV), Classical swine fever virus (CSFV) and a tentative “Giraffe” species (Fauquet et al., 2005). Genetic characterizations of ruminant pestiviruses have shown that BVDV and BDV can cross species barriers and infect a wide range of hosts within the *Artiodactyla* (Nettleton and Entrican, 1995). Phylogenetic analyses of pestivirus isolates originating from sheep and goats revealed that BVDV in sheep and goats is not uncommon (Vilcek et al., 1997; Pratelli et al., 2001; Giangaspero and Harasawa, 2004; Krametter-Froetscher et al., 2008a). In contrast BDV in cattle has only been de-

scribed in a cattle originating from Tyrol, a Federal state of Austria (Hornberg et al., 2009) and in five cases originating from England and Wales (Cranwell et al., 2007; Strong et al., 2009). The BDV isolate described by Hornberg et al. (2009) showed a high similarity at the nucleotide level to the BDV strains detected by Krametter-Froetscher et al. (2007a) in sheep in Vorarlberg a neighbour state of Tyrol.

Epidemiological surveys have demonstrated widespread natural infections with pestiviruses in cattle, sheep and goats in several countries (Loken, 1995; Loken, 2000; Houe, 2005). Epidemiological investigations among breeding cattle, carried out between 1996 and 1998 in Lower Austria, a Federal state in the eastern part of Austria, revealed a prev-

alence of persistently infected cattle herds of 33% (Rossmannith and Deinhofer, 1998).

In Lower Austria, Rossmannith et al. (2005) reported a decrease of the proportion of persistently infected cattle from 0.7% to 0.06% and a decrease of animals pestivirus antibody positive from 24% to 13% between 1999 and 2002 among cattle on common grasslands after a voluntary BVDV control program according the Swedish model was carried out.

Schoepf et al. (2005) described an effective reduction of cattle persistently infected with BVDV from 1.22% to 0.37% between 1999 and 2003 due to a voluntary BVDV eradication regime in Tyrol. Studies of the seroprevalence among the sheep population in Austria have revealed particularly in the mountainous regions where communal Alpine pasturing of cattle, sheep and goats is an important part of farming individual prevalences up to 68% and herd prevalences up to 89%. (Krametter-Froetscher et al., 2007b). In Austria in sheep marked geographical variations, attributed to differences in farming practices, animal density and geography were described (Krametter-Froetscher et al., 2007b).

During an epidemiological investigation carried out in Vorarlberg in 2003, BDV has been isolated for the first time from sheep in Austria (Krametter-Froetscher et al., 2007a). In a further study nine sheep clinically healthy and with BDV persistently infected were found in Vorarlberg (Krametter-Froetscher et al., 2009). The first outbreak of Border disease in a sheep flock in Austria, mainly characterized by abortion was described in 2008 on a farm housing sheep and cattle. In this case the source of infection was a new, clinically healthy breeding ram introduced into the sheep herd (Krametter-Froetscher et al., 2008c).

Clinical cases of Border Disease in sheep have been described from several neighbour countries of Austria such as Switzerland, Italy and Germany (Cravero et al., 1975; Pratelli et al., 1999; Schaarschmidt et al., 2000; Braun et al., 2002).

In the early 1990s, when the crucial epidemiological role of persistently BVDV infected cattle has been demonstrated, several European countries have initiated national and regional control and eradication programs. The Scandinavian countries were pioneers to attempt a control of new infections in 1993–1994 (Sandvik, 2004; Houe et al., 2006). In 1997, Austria followed with regional voluntary programs, especially among breeding cattle (Obritzhauser et al., 2005; Rossmannith et al., 2005; Schoepf et al., 2005).

In August 2004, a national BVDV control program has been implied by law in Austria with the goal to identify and to cull persistently infected cattle. If successful, these control measures should result in a zero seroprevalence in cattle in the nearer future.

Previous epidemiological studies have shown that in Austria in sheep a pestivirus epidemiology independent from cattle exists. Moreover, Krametter-Froetscher et al. (2008b) described the transmission of BDV from sheep naturally persistently infected with BDV to cattle. In a further study a possible transmission of BDV from sheep to cattle during an BD outbreak among the sheep on a farm housing sheep and cattle was described in Austria (Krametter-Froetscher et al., 2008c).

The aim of this study was to describe on one hand the success of the BVDV control program implemented in cattle in 2004 and on the other hand we indicate that the outcome of the BVDV program is compromised without a pestivirus control program among the sheep population in several regions of Austria and under particularly farming practices.

MATERIAL AND METHODS

Generally the Austrian sheep population comprises 333 000 sheep out of 14 655 flocks, with a high sheep density in the mountainous parts of Austria. During summertime 115 864 sheep graze on communal alpine pastures in the mountainous regions of Austria. In the large sheep breeding areas like Tyrol 67 650 sheep (whole sheep population is 75 700) share alpine pastures with possible contact to cattle especially on watering places (313 144 hectares of 435 444 hectares agricultural areas are alpine meadows) during summertime. Similar situations are seen in Vorarlberg, western Carinthia, large areas in the south and west of Salzburg and several parts of Styria. In Austria 272 046 suckler cows and 55 993 dairy cows share alpine meadows during summertime. 110 462 of the described cattle graze on alpine meadows in Tyrol. In Austria at least 6000 farmers keep sheep and cattle on the same farms. In 2008 Austria exported 9 542 sheep and imported 2 765 sheep. Austria also imported 34 176 breeding cattle and exported 66 090 breeding cattle (BMLFUW, 2008).

The Austrian sheep and cattle population is officially free of Scrapie, *Mycobacterium bovis*, Rabies, Aujeszky's disease, *Brucella abortus*, *Brucella melitensis*, Enzootic Bovine Leukosis, Bovine

Herpesvirus-1 and Foot and Mouth Disease. Within the annually investigations carried out in cattle between 2001 and 2008, six cattle were tested positive for BSE. Among sheep in Austria in 2008 four cases of *Brucella ovis* were documented. In sheep, goats and cattle, clinical cases of Johne's disease are since 2006 notifiable in Austria. In 2008 Johne's disease was found in 38 cattle out of 29 farms. In 2008 in Austria only one case of *Campylobacter fetus* subsp. *venerealis* in cattle was announced. In 2008, BVDV was diagnosed on 175 cattle farms in Austria (AGES, 2008).

Epidemiological study

Animals and blood collection. Located in the East of Austria, the Clinic of Ruminants at the Veterinary Hospital Vienna mainly treats animals originating from the eastern provinces. Between August 2004 and December 2007 a total of 1307 cattle were admitted, 948 from Lower Austria, 181 from Styria, 87 from Burgenland, 75 from Upper Austria, 8 from Carinthia, 7 from Salzburg and one animal from Vienna. Geographical origin, herd and individual informations of all cattle were recorded. According to the local veterinarians, all cattle admitted, originated from farms with the BVDV program state "free of BVDV".

Antibody detection. Blood samples collected of all animals were tested for antibodies to pestiviruses using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Svanovir, BVDV-Ab; Svanova Biotech AB, Uppsala, Sweden) according to the manufacturer's instructions. Samples with a corrected optical density (OD_{corr}) value < 0.25 were considered negative, samples with an OD_{corr} of ≥ 0.25 as positive.

Antigen detection and RT-PCR analysis. A BVDV Antigen ELISA (HerdCheck BVDV Ag/Serum Plus, IDEXX GmbH, Germany) was performed on all blood samples according to the manufacturer's instructions. All results of the Antigen ELISA were verified by RT-PCR as described by Vilcek et al. (1994).

Pestivirus investigation in a mixed herd (cattle and sheep)

Animals and blood collection. The herd described was located in the mountainous region

of Salzburg, one of nine Federal states of Austria. Sheep and cattle of the home farm had intensive contact throughout the year. Further the sheep herd grazed on a communal alpine pasture until September 2006 with direct and close contact to sheep from other herds. Since 2002 the owner of the cattle herd had participated in a voluntary BVDV program and since 2004 the cattle herd was "free of BVDV" according the national BVDV program. All tests for BVDV specific antibodies had been negative since 2006.

Beginning in 2008 the BVDV eradication in Salzburg has been based on the analysis of ear tissue samples obtained in the course of the earmarking of newborn calves. Using this method, in August 2008 a newborn female calf of the herd described was identified as pestivirus antigen positive (HerdChek BVDV Ag/Serum Plus; IDEXX GmbH, Germany). The calf was too small for its age, but no further clinical abnormalities were recognized by the local veterinarian.

Blood samples were drawn by jugular vein puncture two weeks and four weeks after birth for validation of the diagnosis and for further investigations.

Blood samples were also collected from the mother of the calf, from four contact cattle, as well as from all nine sheep housed on the farm.

Antigen detection. For antigen detection performed on the blood samples from the calf drawn two and four weeks after birth and blood samples from the mother of the calf and the four contact cattle described, served a commercially available ELISA (HerdChek BVDV Ag/Serum Plus; IDEXX GmbH, Germany).

RT-PCR. RT-PCR was carried out on lysed PBMCs (peripheral blood mononuclear cell pellets) of all sheep blood samples, on the blood sample drawn four weeks after birth and organ samples of the calf (lung, kidney, spleen and liver) available after culling (Krametter-Froetscher et al., 2007a). The primers 324 and 326 (Vilcek et al., 1994) amplify a conserved domain at the 5'-end of the viral genome of 288 bp in length.

Sequencing of PCR products. Amplified DNA was extracted using a commercially available kit (QIAquick, PCR purification Kit, Qiagen, Hilden, Germany) following the manufacturer's instructions and served as template for sequencing PCR. The latter was carried out in a volume of 20 µl with a ready to use sequencing PCR mixture (DNA Sequencing Kit, Applied Biosystems). Sequencing

PCR products were analysed with the ABI Prism 310 Genetic Analyser.

Antibody detection. For both the detection of BDV- and BVDV-Ab commercially available ELISA Kits were used (Svanovir, BVDV-Ab, BDV-Ab; Svanova Biotech AB, Uppsala, Sweden). Samples with a corrected optical density (OD corr) value < 0.25 were considered negative.

Sheep samples were also tested by virus neutralisation test (VNT) for antibodies against the BVDV-1 strain NADL and the BDV strain Moredun as described by Krametter-Froetscher et al. (2005).

RESULTS

Epidemiological study

Antibody detection. An overall seroprevalence of 12.3% was detected among the patients of the Clinic for Ruminants investigated between 2004 and 2007. In detail these seroprevalences were 19.3% in Styria, 12.5% in Carinthia, 11.7% in Lower Austria, 9.1% in Burgenland, 7.9% in Upper Austria and 0% in Vienna and Salzburg, respectively.

In Lower Austria seroprevalences increased from 11.9% in 2004, 12.9% in 2005 to 14.3% in 2006 and dropped to 7.4% in 2007. In the other regions a seroprevalence of 17.6% in 2004 and of 17.8% in 2005 decreased to 11.8% in 2006 and 12.2% in 2007. As expected, the prevalence was lower in younger animals with 8.2% in the group < 1.5 year ($n = 426$) and with 6.8% in the group 1.5 to 4.5 years ($n =$

474). In the age group 4.5 to 7.5 years ($n = 308$) a seroprevalence of 19.8% and in the group > 7.5 years ($n = 99$) of 33.3% was detected.

Antigen detection – RT-PCR analysis. All 1307 samples tested negative for both pestivirus antigen and specific RNA.

Pestivirus investigation in a mixed herd (cattle and sheep)

Antigen detection. Pestivirus specific antigen was detected in both blood samples of the calf drawn two and four weeks after birth. The mother and the four contact animals tested negative.

RT-PCR. The blood sample of the calf collected four weeks after birth as well as lung, kidney and spleen tested positive for pestivirus specific RNA, all sheep samples were negative.

Sequence analysis. Sequence analysis revealed identities of 99% (2 nt difference of 217 bp) to Austrian BDV sequences and of 98% (6 nt difference) to reference strain Gifhorn (GenBank Acc.nr. EU637007, pos. 1-217). According to these results, the pestivirus sequence detected in the PI calf was identified as BDV-3.

Antibody detection. Of the six bovines, the mother of the PI calf and three contact animals were positive by BVDV-Ab ELISA (ODcorr values between 0.67–0.86).

Of the sheep, 7 of 9 samples were positive in the BDV-Ab ELISA, all samples were negative in the BVDV-Ab ELISA. By VNT 8/9 samples were posi-

Table 1. Antibody titres of the serum samples of the sheep investigated in the VNT's

Sample number	Age	Ab-titre against BDV	Ab-titre against BVDV-1
1	6 years	1 : 180	1 : 256
2	6 months	1 : 180	1 : 180
3	4 years	1 : 90	1 : 32
4	4 years	1 : 90	1 : 64
5	3 years	1 : 180	1 : 128
6	4 years	1 : 180	1 : 90
7	6 months	negative	negative
8	6 months	1 : 180	1 : 11
9	6 months	1 : 45	1 : 4

BDV = border disease virus, BVDV = bovine viral diarrhoea virus, RT-PCR = reverse transcriptase polymerase chain reaction, RNA = ribonucleic acid, ELISA = enzyme-linked immunosorbent assay

tive, four samples showed a noticeable higher titre to BDV Moredun than to BVDV-1 (Table 1).

DISCUSSION

The study documented here showed a considerable decrease of the pestivirus seroprevalence of cattle investigated after the implementation of the BVDV eradication program. The seroprevalence decreased in Lower Austria from 12.9% in 2004 to 7.4% in 2007 and from 17.6% to 12.2% in the other regions. As a consequence of the voluntary BVDV eradication program established according to the Swedish model in 1996 in Lower Austria, the latter was the region with the lowest seroprevalence. Earlier studies in Lower Austria by Rossmann et al. (2005) detected comparable reductions of seroprevalence rates from 24% in 1999 to only 13% in 2002. In accordance to the seroprevalence, the prevalence of persistently infected animals also decreased considerably, in Tyrol from 1.22% in 1999, 0.37% in 2003 to 0.04% in 2006 and in Lower Austria to 0.11% in 2005 (Schoepf et al., 2005; Oettl et al., 2008; Rossmann et al., 2008). In Styria in 2006 Obritzhauser and Fuchs (2008) found a rate of “farms free of BVDV” of 93.4%. In the 1 307 blood samples available for investigation, no pestivirus specific RNA was detected, possibly due to the very low prevalence and to the fact that local veterinarians are only permitted to refer cattle to the Clinic of Ruminants from farms with the state “BVDV free”.

Serological investigations carried out between 2001 and 2003 among sheep in Austria revealed that BVDV has been widespread. Additionally in Austria, in sheep flocks with cattle contact significantly higher individual and herd prevalence rates among sheep were found compared to sheep and sheep herds without cattle contact (Krametter-Froetscher et al., 2007b). In view of these facts Krametter-Froetscher et al. (2007b) described that the main virus reservoir for pestivirus infection in sheep in Austria are cattle, but locally there exists also a virus reservoir of Border disease virus among sheep independent from cattle. Particularly in the mountainous regions, where communal alpine pasturing is a century old farming practice high seroprevalence levels in sheep were found in a survey carried out between 2001 and 2003 (Krametter-Froetscher et al., 2007b). The first cases of sheep persistently infected with Border disease virus in Austria were found in the alpine region of Vorarlberg in 2003 (Krametter-Froetscher et al., 2007a). The

animals described were clinically healthy and persistently infected with a BDV-3 strain closely related to BDV-3 type species Gifhorn. BDV-3 isolates similar to the BDV-3 strains described in Austria were also found in sheep in Germany (Becher et al., 1999) and Switzerland (Stalder et al., 2005).

In 2008, Krametter-Froetscher et al. (2008c) identified a persistently BDV-3 infected breeding ram in a mixed flock in Salzburg, the cattle herd was “BVDV free”. The ram caused a Border disease outbreak in the sheep mainly characterised by abortion. On the farm described by Krametter-Froetscher et al. (2008c), one cow previously negative to antibodies against pestiviruses seroconverted with an Ab-titer of 1 : 56 to BDV and of 1 : 7 against BVDV. As a consequence, the farm lost the BVDV free status and expensive follow up examinations were necessary. The calf persistently infected with BDV-3 originated like the ram described above, from the same county. Moreover, sequence analysis of the Border disease virus isolated from the calf described here showed a high similarity to the pestivirus isolate of the infected ram described by Krametter-Froetscher et al. (2008c). Hornberg et al. (2009) described in a retrospective study for the first time BDV-3 in a cattle in Tyrol, a neighbour state of Salzburg and Vorarlberg. However, BDV in cattle was uncommon and other than that mentioned by Hornberg et al. (2009), BDV in cattle has only been described in England by Cranwell et al. (2007) and Strong et al. (2009).

Hornberg et al. (2009) suspected sheep as the source of infection of the cattle described. The presumption is sustained by the results of this and of previous investigations (Krametter-Froetscher et al., 2008b,c). In the mixed flock described here all sheep except for one six month old lamb, showed antibody titres against pestiviruses. Moreover in four sheep the titre was higher to BDV strain Moredun than to BVDV-1 strain NADL. Additionally, the cattle herd had been free of antibodies to pestiviruses in the previous years. Therefore in the case described here the reservoir for the reintroduction of pestivirus infection in the cattle herd had been in all probability the sheep herd. Prior to the implementation of the BVDV eradication program, several studies about the genetic diversity of pestiviruses circulating in Austria only detected BVDV-strains in cattle (Vilcek et al., 2001, 2003; Kolesarova et al., 2004). With the eradication of BVDV and the resulting predominance of BDV, the pestivirus epidemiology in Austria possibly change, first evidence is given by Krametter-Froetscher et

al. (2008c) and Hornberg et al. (2009) who detected cattle persistently infected with BDV.

The epidemiological role of these BDV-PI cattle still has to be clarified, in particular whether these animals represent epidemiological dead ends or a continuous source of infection. This study and previous studies (Krametter-Froetscher et al., 2007a,b, 2008b,c; Hornberg et al., 2009) make clear that in Austria particular in regions with communal alpine pasturing and on farms with close contact of cattle and sheep a high risk factor for reintroduction of pestiviruses in BVDV free cattle herds exists, associated with expensive financial penalties to the farmers. However, in consideration of the fact that large sheep breeding countries like UK or New Zealand have only voluntary BVDV eradication programs among cattle and they also use vaccines against BVDV in cattle (Reichel et al., 2008; Heffernan et al., 2009), sheep persistently infected with BDV are inconsiderable for the pestivirus epidemiology there and an investigation of the sheep population for BDV in those countries would be uneconomic. In spite of this fact Strong et al. (2009) suggest a monitoring of the BDV status in sheep that may be in contact with cattle in areas with organised BVD control programmes in the UK.

In contrast to the UK and New Zealand, in Austria the BVDV eradication among cattle is severe controlled by the law and especially for cattle breeder the occurrence of a persistently infected cattle or the sudden occurrence of antibodies to pestiviruses in one of the animals of the herd have serious consequences for the cattle herd (BMG, 2008). Traditionally, in the mountainous regions of Austria numbers of cattle breeder are located and to prevent the reintroduction of Pestiviruses in these herds through contact with persistently infected sheep a monitoring of the BDV status in these areas is needed.

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