First descriptive 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

R. Krametter-Froetscher[1], C. Schmitz[2], V. Benetka[4], Z. Bago[3], K. Moestl[4], E. Vanek[3], W. Baumgartner[1]

[1]Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria
[2]Schlossstraße 17, 5550 Radstadt, Austria
[3]Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Moedling, Austria
[4]Department for Pathobiology, University of Veterinary Medicine, Vienna, Austria

ABSTRACT: Described is the first outbreak of Border disease in a sheep flock in Austria and its impact on the cattle housed on the same farm. Border disease virus infected sheep are a high risk for pestivirus introduction in susceptible cattle herds and should therefore induce a complete revision of the Austrian Bovine viral diarrhea virus program.

Keywords: Border disease; sheep; cattle; Austria

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, **VNT** = virus neutralisation test

Border disease is a congenital viral disease of sheep and goats and was first reported in 1959 in the Border region of Wales and England (Hughes and Kershaw, 1959). The disease is characterised by barren ewes, abortion, stillbirth and the birth of small, weak lambs showing tremor, abnormal body conformation and hairy fleeces (Nettleton et al., 1998). Monies et al. (2004) documented in lambs persistently infected with Border disease virus (BDV) an enteric disease characterised by diarrhoea and illthrift. Serological investigations have shown a worldwide distribution of Border disease virus. Seroprevalence rates vary in sheep from 5 to 50% depending on country or region investigated (Nettleton et al., 1998). Serological investigations in Austria have shown a mean flock prevalence of 62.9% and a mean individual prevalence of 29.4% with marked regional differences (Krametter-Froetscher et al., 2007a). Clinical cases of the disease have been reported from several European countries (Cravero et al., 1975; Pratelli et al., 1999; Schaarschmidt et al., 2000; Braun et al., 2002; Monies et al., 2004). Although in Austria typical clinical Border disease has to date not been reported, Krametter-Froetscher et al. (2007b) described the first cases of sheep persistently infected with Border disease virus. These sheep have been identified during an epidemiological study car-

ried out in the alpine region of Austria and they were clinically healthy. Very recently, Krametter-Froetscher et al. (2008) were able to prove seroconversion of susceptible calves after contact to persistently Border disease virus infected sheep. This communication describes the first cases of clinical Border disease in sheep in Austria. The animals were originated from a mixed farm housing cattle and sheep. All animals of the cattle herd have been routinely tested in the legally implied Bovine viral diarrhea virus (BVDV) eradication program and the herd certified as "Bovine viral diarrhea virus free". The sudden inexplicable seroconversion in the cattle herd led not only to the loss of the "Bovine viral diarrhea virus free" state but to the discovery of Border disease virus in the contact sheep as the most probable source on infection.

Case presentation

The farm described here is located in Salzburg, one of nine Federal states of Austria. This part of Salzburg is mountainous and communal alpine pasturing of sheep, goats and cattle is a century old farming practice in this region. During wintertime cattle and sheep from the farm described, were housed in the same stable, but in separate ranges. From June until September the sheep shared alpine meadows with sheep and heifers from different farms. Heifers from the farm documented here grazed on alpine meadows with cattle from other farms. Since autumn 2005 the cattle herd of the documented farm was certified according the Bovine viral diarrhea virus eradication program as "free of Bovine viral diarrhea virus", the same was true for cattle sharing the alpine meadows with the documented herd. An investigation of the Bovine viral diarrhea-status of the herd in October 2007 confirmed the negative status. In the following month the cattle had only contact to the sheep of the homefarm. At the end of October 2007 a new breeding-ram was introduced into the sheep herd. The ram was a two years old mountain sheep and originated from the neighbour county. The ram was bought on an auction and achieved all medical and breeding obligations requested in the Federal state Salzburg.

In December 2007 the first abortion was recognised in an ewe. At this time the sheep breeding stock of the farm consisted of 11 ewes and the ram described above. From December 2007 until February 2008 abortion was documented in further seven of the ewes. The most obvious features of the aborted fetuses were changes in the fleece. Some had the typical "hairy fleece", others had no haircoat at all. Moreover, mummified fetuses were also seen. In February 2008 the local veterinarian sent material from a further abortion (two fetuses and placentas) to the Austrian Agency for Health and Food Safety in Moedling. At that time 8 out of 11 ewes had aborted, the remaining three (following a normal pregnancy) gave birth to in total five clinically healthy lambs. Clinically signs aside from abortion were not recognized in the sheep flock described.

Histological and virological (RT-PCR and sequence analysis) investigations performed on the organs of the fetuses and the placentas revealed that the cause of abortion was Border disease virus. Immediately following this diagnosis blood samples (two vials, with and without anticoagulant) from the sheep (11 ewes, one buck, five lambs) of the farm were collected. All samples were analysed for pestivirus specific RNA using RT-PCR (Vilcek et al., 1994). The ram, a two year old mountain sheep was detected as infected with Pestivirus. By retesting the ram two weeks later a persistent pestivirus infection was confirmed. For Border disease virus genotyping, pestivirus positive samples were submitted to RT nested PCR as described by Vilcek et al. (1997) and Becher et al. (1999). Sequencing of 452 bp of the Npro region of the amplification products obtained of the sample of the ram (leucocyte pellet) and of one aborted fetus revealed that the two samples were to 100% identical to each other and to 94% with Border disease virus-3 type species Gifhorn (GenBank acc. No. AY163653). Identities of 89–92% were found to various french and swiss isolates classified in the Border disease virus-3 genotype (France: EF693964: 91%, EF693966: 90%, EF693967: 91%, EF693969: 91%, EF693970: 90%; Switzerland: AY895008: 89%, AY895009: 92%).

To determine the antibody prevalence rates among the sheep in this flock a commercially available enzyme-linked immunosorbent assay (Svanovir® BDV-Ab ELISA; Svanova, Biotech AB, Uppsala, Sweden) and virus neutralisation tests (VNTs) (BVDV-1 strain NADL, BDV strain Typ 137/4) were carried out (Krametter-Froetscher et al., 2005). The ram persistently infected was negative in the ELISA as well as in the VNTs. In the Border disease virus-ELISA of the 16 samples of the ewes and lambs 14 samples were positive, one

sample negative and one sample was questionable. The results of the VNTs showed that all 16 samples were positive. 15 samples showed clearly higher titres to the Border disease virus strain, only one sample revealed the same titres against the Bovine viral diarrhea virus strain and the Border disease virus strain. Seven samples showed a titre of 1 : 640 or higher against the Border disease virus strain. The lowest Border disease virus-antibody-titre was 1 : 80 in two samples. Investigations of the Bovine viral diarrhea status in the cattle herd showed that one cow born in 2004 obviously has seroconverted (positive in the Svanovir® BDV-Ab ELISA, Svanova, Biotech AB, Uppsala, Sweden as well as in the Svanovir® BVDV-Ab ELISA, Svanova, Biotech AB, Uppsala, Sweden). In the VNT's this serum showed a titre of 1 : 7 to the Bovine viral diarrhea virus strain and 1 : 56 to the Border disease virus strain. The Bovine viral diarrhea-status of the other animals of the herd had not changed. The cow which had seroconverted had aborted on the 15th of March. The fetus and the placenta had been sent to the Austrian Agency for Health and Food Safety in Moedling for further investigations. The fetus with a crown-rump length of 18 centimetres and the placenta were totally mummified. Routine diagnosis carried out on the fetus and the placenta revealed no clear cause of abortion. Pestivirus specific RNA was not detected in the fetus or placenta. The ram described above was slaughtered, after the diagnosis of Border disease virus infection was confirmed.

DISCUSSION

Clinical signs as a consequence of a Border disease outbreak have never been reported in Austria, although previous epidemiological studies had shown that Border disease virus infection is common in Austrian sheep herds (Krametter-Froetscher et al., 2007a) and persistent Border disease virus infection had been described in healthy sheep in Vorarlberg, a Federal State of Austria west of Salzburg (Krametter-Froetscher et al., 2007b). The persistently Border disease virus infected ram described in this study was two years old and clinically healthy. Sequence analysis in the N^{pro} region of the Border disease virus isolate revealed a high similarity to the Border disease-viruses described by Krametter-Froetscher et al. (2007b) in Vorarlberg and to isolates documented by Braun et al. (2002) from Switzerland. Starting two months after the ram's introduction in the mixed sheep/cattle herd a serie of abortion started (8 of 11 ewes and one cow). Considering the known Bovine viral diarrhea virus free status of the cattle herd until the introduction of the infected ram, the clinical outcome and the seroconversion in the cow which aborted, it has to be assumed that the persistently Border disease virus infected ram had introduced the infection not only into the sheep population of the farm but also into the cattle herd, that caused seroconversion of a seronegative and therefore susceptible cow. This assumption is confirmed by the results of the VNTs, with a titre of 1 : 56 against Border disease virus and of only 1 : 7 against Bovine virus diarrhea virus, possibly a cross reaction of the Border disease virus-specific antibodies. Although the cause of abortion in the cow could not be diagnosed (most probably because of the very poor condition of the aborted fetus and the placenta), Border disease virus may have been the causative agent.

This case of transmission of Border disease virus from sheep to a cow is proofed by our recently published study, where we were able to show that under a field situation calves in close contact to clinically healthy sheep persistently infected with Border disease virus-3, seroconverted between Day 28 and Day 51 after exposure (Krametter-Froetscher et al., 2008). Cranwell et al. (2007) also reported the detection (by RT-PCR) of three Border disease virus infected bovines in England and Wales.

If seroconversion against pestiviruses is detected in a certified "Bovine viral diarrhea virus free" cattle herd the herd loses its "Bovine viral diarrhea virus free status" and expensive follow up examinations have to be performed. Furthermore, for various purposes like transport, and for the disposition of breeding cattle, an individual certification assuring the Bovine viral diarrhea virus negative status of this animal is needed.

In several years Austria's cattle population will be free of Bovine viral diarrhea virus and also free of antibodies against Bovine viral diarrhea virus. This condition will be the consequence of the Austrian Bovine viral diarrhea virus eradication program (implemented in August of 2004) which is characterised by the identification and eradication of persistently infected cattle. In 2004 little and very controversial information was available about sheep as a source of pestivirus infection for cattle; small ruminants had therefore not been included in the planning and implementation of the national eradi-

cation program. However, this case report as well as previous studies (Krametter-Froetscher et al., 2008) now make clear that Border disease virus infected sheep are a high risk for pestivirus introduction in susceptible cattle herds and should therefore induce a complete revision of the Austrian Bovine viral diarrhea virus program as soon as possible.

Acknoledgements

The authors are grateful to Dr. Christian Simmerstaetter, Dr. Eveline Wodak and Bauer Christian for collaboration.

REFERENCES

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

Braun U., Hilbe M., Ehrensperger F., Salis F., Alther P., Strasser M., Stalder H.P., Peterhans E. (2002): Border Disease in einem Schafbetrieb. Schweizer Archiv fur Tierheilkunde, 144, 419–426.

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

Cravero G.C., Fatzer R., Frankhauser R. (1975): Border Krankheit (Hypomyelinogenesis congenital) bei Lammern in der Schweiz. Schweizer Archich fur Tierheilkunde, 117, 119–121.

Hughes L.E., Kershaw G.F. (1959): "B" or Border disease, an undescribed disease of sheep. Veterinary Record, 71, 313–317.

Krametter-Froetscher R., Loitsch A., Moestl K., Sommerfeld-Stur I., Baumgartner W. (2005): Seropravalenz von Border disease und Boviner Virusdiarrho bei Schafen und Ziegen in ausgewahlten Regionen Osterreichs. Veterinary Medicine. Austria/Wien. Tierarztliche Monatsschrift, 92, 238–244.

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

Krametter-Froetscher R., Kohler H., Benetka V., Moestl K., Golja F., Vilcek S., Baumgartner W. (2007b): 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., Benetka V., Moestl K., Baumgartner W. (2008): Transmission of Border Disease Virus from sheep to calves – a possible risk factor for the Austrian BVD eradication programme in cattle? Wiener Tierarztliche Monatsschrift/Veterinary Medicine Austria, 95, 200–203.

Monies R.J., Paton D.J., Vilcek S. (2004): Mucosal disease-like lesions in sheep infected with Border disease virus. Veterinary Record, 155, 765–769.

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

Pratelli A., Bollo E., Martella V., Guarda F., Chiocco D., Buonavoglia C. (1999): Pestivirus infection in small ruminants: virological and histopathological findings. Microbiologica. 22, 351–356.

Schaarschmidt U., Schirrmeier H., Strebelow G., Wolf G. (2000): Nachweis von Border Disease Virus in einem Schafbestand in Sachsen. Berliner Munchner Tierarztlichen Wochenschrift, 113, 284–288.

Vilcek S., Herring A.J., Herring J.A., Nettleton P.F., Lowings J.P., 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 P.F., Paton D.J., Belak S. (1997): Molecular characterization of ovine pestiviruses. Journal of General Virology, 78, 725–735.

Received: 2008–10–13
Accepted after corrections: 2008–11–18

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