Vet. Med. Austria / Wien. Tierärztl. Mschr. **95** (2008), 200 - 203

From the Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health[1], and the Clinical Virology, Clinical Department for Diagnostic Imaging, Infectious Diseases and Clinical Pathology[2], University of Veterinary Medicine Vienna

Transmission of *Border Disease Virus* from sheep to calves - a possible risk factor for the Austrian BVD eradication programme in cattle?

R. KRAMETTER-FRÖTSCHER[1], V. BENETKA[2], K. MÖSTL[2] and W. BAUMGARTNER[1]

eingelangt am 6.12.2007
angenommen am 18.2.2008

Keywords: *Pestivirus*, sheep, cattle, epidemiology, BVD eradication.

Summary

Infections of cattle with *bovine viral diarrhoea virus* (BVDV) are of great economical importance. Therefore in Austria like in other countries a BVD eradication programme in the cattle populations has been implemented in 2004. BVDV is closely related to *Border Disease* V*irus* (BDV) causing disease in sheep and goats. Both viruses belonging to the genus *Pestivirus* are known to be of limited species specificity, but no detailed information is available about BDV transmission from sheep to cattle under field conditions. In the present study 4 two-month old Pestivirus-negative calves were kept together with 6 healthy, persistently BDV infected sheep allowing natural contact between the animals and thereby imitating field conditions. Seroconversion of all 4 calves against BDV confirmed the successful virus transmission from the sheep to the calves. Previous studies in Austria revealed a high Pestivirus seroprevalence in the sheep populations investigated. In the light of the results of this study that persistently infected sheep easily transmit BDV to cattle and because of the common practice of keeping sheep and cattle together and of possible contacts on alpine pastures during summer time further investigations are urgently needed to clarify this crucial factor eventually endangering the success of the Austrian BVD eradication programme.

Abbreviations: BDV = *Border disease virus*; BVD = bovine viral diarrhoea; BVDV = *bovine viral diarrhoea virus*; COD = corrected optical density; ELISA = Enzyme-Linked Immunosorbent Assay; PI = persistently infected; PBMCs = Peripheral Blood Mononuclear Cells; RNA = Ribonuleic Acid; RT-PCR = Reverse Transcriptase Polymerase Chain Reaction; VN = virus neutralisation; VT = Versuchstier (experimental animal)

Schlüsselwörter: *Pestivirus*, Schaf, Rind, Epidemiologie, BVD-Eradikation.

Zusammenfassung

Übertragung von *Border Disease Virus* von Schafen auf Kälber - ein möglicher Risikofaktor für die BVD-Bekämpfung bei Rindern in Österreich?

Infektionen von Rindern mit dem *Bovinen Virusdiarrhövirus* (BVDV) sind von großer wirtschaftlicher Bedeutung. Daher wurde in Österreich 2004 wie in anderen Ländern auch ein Bekämpfungsprogramm der BVDV-Infektion in der Rinderpopulation auf Basis einer Verordnung initiiert. BVDV ist nahe mit dem *Border Disease Virus* (BDV) der Schafe und Ziegen verwandt. Beide Erreger gehören dem Genus *Pestivirus* an und weisen nur mäßige Speziesspezifität auf, wodurch Interspezies-Übertragungen möglich sind. Über die Möglichkeit einer BDV-Übertragung vom Schaf auf das Rind unter Feldbedingungen ist allerdings aus der Literatur nur wenig bekannt. Es wurden daher 4 zwei Monate alte, Pestivirus-negative Kälber mit 6 persistent BDV-infizierten, gesunden Schafen mit direkter Kontaktmöglichkeit gemeinsam gehalten, wodurch Feldbedingungen simuliert wurden. Die erfolgreiche BDV-Infektion der Kälber wurde durch Serokonversion aller 4 Kälber gegen BDV nachgewiesen. Nachdem Pestivirusinfektionen in der österreichischen Schafpopulation zumindest regional weit verbreitet sind, Schafe und Rinder oft gemeinsam gehalten werden und die Alpung während der Sommerzeit ein spezielles Infektionsrisiko darstellt, sind weitere Untersuchungen erfoderlich, um die Bedeutung dieses Ergebnisses genauer zu erheben. Im speziellen ist zu untersuchen, ob persistent infizierte Schafe eine Gefahr für den Sanierungserfolg der BVD in den Rinderpopulationen darstellen.

Introduction

The genus *Pestivirus* within the family *Flaviviridae* is divided into 4 accepted species: *border disease virus* (BDV), *bovine viral diarrhoea virus* (BVDV) -1, BVDV-2 and *classical swine fever virus* and a tentative 'Giraffe' species (FAUQUET et al., 2005). Infections with BDV are widespread in most sheep breeding countries throughout the world and BVDV infections are endemic in cattle worldwide; they cause substantial economic losses to the sheep and cattle industries through their impact on reproduction and health (NETTLETON and ENTRICAN, 1995). Border disease in sheep is characterized by barren ewes, abortion, stillbirths, the birth of weak, small and persistently infected lambs, abnormalities of the hair and nervous

tremor (NETTLETON et al., 1998). Persistently infected lambs constantly shed virus and play an epidemiological key role. BVDV and BDV are not strictly host specific and can cross infect cattle, sheep, goats, pigs and non-domesticated species (NETTLETON and ENTRICAN, 1995), whereas *classical swine fever virus* has been isolated only from pigs (HARKNESS, 1985). Sequence analysis of Pestivirus isolates originating from sheep from several countries revealed that both, BVDV and BDV, are endemic in sheep worldwide (VILCEK et al., 1997). CARLSSON and BELAK (1994) described the transmission of Border disease from a persistently infected ewe to cattle. One year later sequence analysis grouped these isolates into the BVDV species (PATON et al., 1995). No field infection of BDV in cattle, due to the spread of the virus from sheep has been documented (NETTLETON and ENTRICAN, 1995). Recently CRANWELL et al. (2007) reported shortly the detection (by RT-PCR) of BDV infected bovines.

Investigations for antibodies against Pestiviruses in sheep in Austria have shown a flock prevalence of 62.9 per cent and an individual prevalence of 20.4 per cent (KRAMETTER-FRÖTSCHER et al., 2007a). Based on comparative neutralisation studies it was shown that the majority of seropositive animals had experienced a BVDV-1 infection. However, BDV circulates in sheep populations in western Austria: 3 healthy, persistently BDV infected sheep (BDV-PI) were detected in 2003 during an epidemiological study on Pestiviruses (KRAMETTER-FRÖTSCHER et al., 2007b).

In Austria, a national BVD eradication programme commenced in the autumn 2004 for cattle according to the Scandinavian model. The goal of this programme is to identify and cull all persistently infected cattle, which happen to be the main source for the spreading of this virus infection. In case of success, the eradication programme will result in a zero seroprevalence and full susceptibility in cattle in several years. The question arises as to whether sheep that are persistently infected with Pestiviruses (occurrence in Austria demonstrated for BDV, no information available for BVDV) may be a source of infection for susceptible cattle, and therefore, pose a danger to the success of the eradication programme.

The present study was performed in order to investigate the possibility of BDV transmission from sheep to cattle by natural contact with BDV-PI sheep, and thereby imitating field conditions, albeit with a high viral load.

Materials and methods

6 healthy sheep had been detected as persistently infected with BDV during surveillance studies in sheep in Austria. Diagnosis was performed by means of repeatedly conducted RT-PCR according to the method described by VILCEK et al. (1994). Sequencing of the amplification products and alignment had revealed highest homologies with different BDV strains available in the GenBank (accession numbers AF026768, U65064, U65063 and AF026769) and to the BDV genotype 3 isolate described earlier from the same region by KRAMETTER-FRÖTSCHER et al. (2007b). The 6 sheep were kept together with 4, 2 month old male calves. The present study was approved by the Ethics Committee of the Veterinary University of Vienna and the Austrian Ministry for Health. All 4 calves originated from a BVDV free cattle herd. Their BVDV status was confirmed by Pestivirus specific RT-PCR according to VILCEK et al. (1994). Virus neutralisation tests for antibodies against Pestiviruses were performed as described by KRAMETTER-FRÖTSCHER et al. (2005). The first blood sampling from the calves was performed at the home farm, and the second sampling on the day of their arrival at the quarantine stable, and immediately before the calves made the first contact with the PI-sheep. Neither Pestivirus specific RNA, nor antibodies to BDV or BVDV were detected in the blood samples of the calves. At the quarantine stable the calves and sheep shared a meadow with an area of 1,500 m^2 and a stable with an area of 55 m^2. During feeding time, the calves and sheep had close contact. During the resting period of the day the calves and sheep preferred different places on the meadow.

Blood samples from the calves were drawn on days 6, 15, 28, 37, 51, and 63 after the initial contact between the sheep and cattle (2 vials, with and without anticoagulant). A commercially available ELISA kit (BDV-Ab; Svanova Biotech, Uppsala, Sweden) for antibodies to ruminant Pestiviruses, which had been evaluated for the use in small ruminants by KRAMETTER-FRÖTSCHER et al. (2005), was used for the detection of Pestivirus antibodies. Sera with a corrected optical density (COD) value <0.250 were considered negative. Moreover, the serum samples of day 63 were tested for neutralising antibodies against 2 cytopathic BVDV-strains (BVDV-1 strain NADL and BVDV-2 strain 125) and against BDV strain Typ 137/4 (KRAMETTER-FRÖTSCHER et al., 2005). Virus neutralising titres of up to 1:2 were considered negative. RT-PCR was used for the detection of Pestivirus specific RNA in PBMCs (VILCEK et al., 1994). Whenever samples were collected, a clinical examination of all 4 calves was performed according to BAUMGARTNER (2005). Behaviour and appetite were monitored daily by the keepers.

Results

During the observation period, no changes in appetite or behaviour were recorded. On the occasions of clinical examinations no abnormalities (increase in body temperature, apathy, rumen stasis) were diagnosed.

All 4 calves seroconverted to Pestiviruses. Using the ELISA kit, antibodies to Pestiviruses were first detected in the samples collected on day 28 in 2 calves (VT 5/06 and 6/06) after the first contact with the PI-sheep. On day 37, the third animal (VT 7/06) had seroconverted and the samples of day 51 were all positive. The samples of day 63 were used for comparative studies for antibodies against 3 Pestivirus species using virus neutralisation tests. In all of the calves, the clearly highest titres were reached against BDV (Tab. 1). Pestivirus specific RNA was not detected in any of the blood samples.

Discussion

The results of the present study show that a BDV infection can be transmitted from PI sheep to susceptible calves. All of the calves that had been tested negative repeatedly for Pestivirus infection, seroconverted after contact with the PI sheep. The clearly highest titres were achieved against BDV compared to BVDV-1 and -2, which is in agreement with the fact that the sheep were persis-

Tab. 1: Serological results on days 15, 28, 37, 51 and 63 after the first contact between the 4 calves and the PI sheep

Animal	VT 4/06	VT 5/06	VT 6/06	VT 7/06
Day 15 ELISA result (OD)	negative (0.085)	negative (0.240)	negative (0.018)	negative (0.165)
Day 28 ELISA result (OD)	negative (0.158)	positive (0.375)	positive (0.576)	negative (0.165)
Day 37 ELISA result (OD)	negative (0.160)	positive (0.291)	positive (0.477)	positive (0.380)
Day 51 ELISA result (OD)	positive (0.379)	positive (0.304)	positive (1.013)	positive (0.499)
Day 63				
VN result BDV	positive 1:128	positive 1:91	positive 1:256	positive 1:181
VN result BVDV-1	positive 1:8	positive 1:11	positive 1:91	positive 1:11
VN result BVDV-2	negative <1:2	positive 1:2.8	positive 1:16	positive 1:11

tently infected with BDV. In none of the blood samples viral RNA was detectable, a result that could be explained by the fact that viremia in a transient infection is usually rather short and weak. It would, therefore, have been necessary to take samples in shorter intervals.

KRAMETTER-FRÖTSCHER et al. (2007a) described the potential role of persistently infected cattle as BVDV reservoirs for sheep in Austria, but investigations conducted throughout several regions in Austria have shown that Pestivirus infections also occur in sheep herds with no contact to cattle. Recently the source of these infections could be identified: persistently with BDV infected sheep (KRAMETTER-FRÖTSCHER et al., 2007a,b).

The central element of the BVD control programme in Austria is the detection and eradication of all persistently infected cattle. In contrast to many European countries (LINDBERG et al., 2006), the use of vaccines is not permitted in Austria. If the Austrian programme is applied successfully, the cattle herds in Austria will be free of BVDV as well as of antibodies against BVDV. The programme will lead to a high susceptibility to Pestivirus infections in the Austrian cattle population. To the authors' knowledge the possible infection of cattle with BDV in a field study has never been described in detail before. CRANWELL et al. (2007), who reported the detection of BDV infected bovines without presenting detailed information point out that those infections may have important implications for BVD control programmes. This may be especially true for the situation in Austria, where on Alpine meadows, cattle, sheep, and goats from different farms have close contact. The risk of BDV infection for seronegative cattle will play a pivotal role in several years. Moreover, in Austria many farms house both, cattle and sheep. In these flocks, cattle that are free of antibodies to Pestiviruses will have a high risk of becoming infected in the course of a purchased clinically healthy sheep that is persistently infected with BDV. In Sweden, sheep persistently infected with BVDV have been described as a possible source for Pestivirus-infection in cattle (CARLSSON and BELAK, 1994; PATON et al., 1995). However, BVDV has not been isolated from sheep up to now in Austria. The impact of BDV infection on the reproductive function in susceptible pregnant cattle still remains unclear. An outcome similar as following BVDV infections could eventually suggest a revision of the eradication programme in Austria. Seroconversions to Pestiviruses in BVDV free herds would lead to restrictions in the transport and disposition of cattle from such a farm. Moreover, expensive investigations according to the BVD eradication programme would have to be repeated.

Acknowledgements

The authors would like to thank Dr. Joachim Weikel, Dr. Eveline Wodak and Christian Bauer from the Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Robert Kochgasse 17, A-2340 Mödling, Austria, for the excellent technical and professional support. Thanks also go to those who assisted with the virological investigations: Mag. Karin Walk, Helga Lussy and Dr. Muna Latif.

References

BAUMGARTNER, W. (2005): Klinische Propädeutik der inneren Krankheiten und Hautkrankheiten der Haus- und Heimtiere. 6. Aufl., Parey, Berlin, S. 18-160.

CARLSSON, U., BELAK, K. (1994): Border Disease Virus transmitted to sheep and cattle by a persistently infected ewe: epidemiology and control. Acta Vet. Scand. **35**, 79-88.

CRANWELL, M.P., OTTER, A., ERRINGTON, J., HOGG, R.A., WAKELEY, P., SANDVIK, T. (2007): Detection of Border disease virus in cattle. Vet.Rec. **161**, 211-212.

FAUQUET, C.M., MAYO, M.A., MANILOFF, J., DESSELBERGER, U., BALL, L.A. (2005): Virus taxonomy, classification and nomenclature of viruses. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London.

HARKNESS, J.W. (1985): Classical swine fever and its diagnosis: current view. Vet. Rec. **116**, 288-293.

KRAMETTER-FRÖTSCHER, R., KOHLER, H., BENETKA, V., MÖSTL, 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 Public

Health **54**, 209-213.
KRAMETTER-FRÖTSCHER, R., LOITSCH, A., KOHLER, H., SCHLEINER, A., SCHIEFER, P., MÖSTL, K., GOLJA, F., BAUMGARTNER, W. (2007a): Serological survey for antibodies against pestiviruses in sheep in Austria. Vet. Rec. **160**, 726-730.
KRAMETTER-FRÖTSCHER, R., LOITSCH, A., MÖSTL, K., SOMMERFELD-STUR, I., BAUMGARTNER, W. (2005): Seroprävalenz von Border disease und Boviner Virusdiarrhö bei Schafen und Ziegen in ausgewählten Regionen Österreichs. Vet. Med. Austria / Wien. Tierärztl. Mschr. **92**, 238-244.
LINDBERG, A., BROWNLIE, J., GUNN, G.J., HOUE, H., MOENNIG, V., SAATKAMP, H.W., SANDVIK, T., VALLE, P. S. (2006): The control of bovine viral diarrhoea virus in Europe: today and in the future. Rev. Sci. Tech. Off. Int. Epizoot. **25**, 961-979.
NETTLETON, P.F., ENTRICAN, G. (1995): Ruminant Pestiviruses. Br. Vet. J. **151**, 615-642.
NETTLETON, P.F., GILRAY, J.A., RUSSO, P., DLISSI, E. (1998): Border disease of sheep and goats. Vet. Res. **29**, 327-340.
PATON, D.J., CARLSSON, U., LOWINGS, J. P., SANDS, J.J., VILCEK, S., ALENIUS, S. (1995): Identification of herd-specific bovine viral diarrhoea virus isolates from infected cattle and sheep. Vet. Microbiol. **43**, 283-294.
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. Arch. Virol. **136**, 309-323.
VILCEK, S., NETTLETON, P.F., PATON, D.J., BELAK, S. (1997): Molecular characterization of ovine pestiviruses. J. Gen. Virol. **78**, 725-735.

Authors' address:
Dr. Reinhild Krametter-Frötscher, Dr. Viviane Benetka, Univ.Prof. Dr. Karin Möstl, Univ.Prof. Dr. Walter Baumgartner, Veterinärplatz 1, A-1210 Vienna, Austria.
e-Mail: Reinhild.Krametter@vu-wien.ac.at

Atlas der Anatomie des Hundes. Lehrbuch für Tierärzte und Studierende. Von K.-D. BUDRAS, W. FRICKE und R. RICHTER. Schlütersche Verlagsgesellschaft, Hannover, 2007. 8. überarb. Aufl., 228 Seiten, 71 großformatige Abbildungen. EUR 76,-, ISBN 978-3-89993-039-9.

Der „Atlas der Anatomie des Hundes" setzt auch in der aktuellen, teilweise überarbeiteten 8. Auflage das bewährte Konzept dieses Anatomieatlanten als Standardwerk und unentbehrliches Grundlagenwissen für Studium und Praxis fort. Die Neuauflage wurde komplett durchgesehen und in Text und Abbildungen optimiert. Insbesondere das bewährte didaktische Konzept einer Gegenüberstellung von deskriptiven und illustrativen Elementen auf gegenüberliegenden Buchseiten ermöglicht eine integrative Wissensvermittlung. Die topographische Anatomie ist in 11 Kapitel unterteilt: Körperoberfläche und Stammskelett, Hals und Brustregion, Schultergliedmaße, Brust- und Bauchwand, Brusthöhle, Bauchhöhle, Harn- und Geschlechtsorgane inklusive Becken, Beckengliedmaße, Kopf, Zentralnervensystem sowie Sinnesorgane. Die Abbildungen sind von bestechender Qualität, detailliert und konsequent beschriftet, ohne beim Betrachten unübersichtlich zu wirken und fördern ein besseres Verständnis der anatomischen Zusammenhänge. Zusätzlich findet sich eine umfangreiche Beschreibung ausgewählter Themengebiete in einem weiteren Kapitel in Form eines tabellarischen Teiles zur speziellen Anatomie. In der Myologie werden die Muskelgruppen bezeichnet und jeder Muskel mit Namen, Ursprung, Ansatz, Innervation, Funktion und besonderen Anmerkungen, wie z.B. Ausnahmen und Faserverläufen beschrieben. In der Lymphologie sind alle Lymphzentren angeführt, unter besonderer Berücksichtigung der zugehörigen Lymphknoten, ihrer Lage, dem tributären Gebiet und dem Abfluss. Weiterführend finden sich besondere Anmerkungen hinsichtlich ihrer Topographie, Form, Anzahl, Regelmäßigkeit des Auftretens und Palpierbarkeit. Bei den Gehirnnerven werden deren Namen und die ihrer Äste, die Faserqualitäten, die jeweiligen Innervationsgebiete als auch die Ursprünge und Schädelaustritte ausführlich dargestellt. Zur Veranschaulichung der Muskeln, Lymphzentren und Nerven ist (sind) konsequent die zugehörige(n) Abbildung(en) im topographischen Teil des Atlas angegeben, wodurch ein Auffinden im Werk wesentlich erleichtert wird. Etwa 20 Seiten des Werkes sind überraschend detailliert auch der allgemeinen Anatomie gewidmet, wobei, wie schon bei der vorangegangenen Auflage angeregt, ein Voranstellen dieser Thematik an den Beginn des Atlanten durchaus sinnvoll gewesen wäre. Besonders wertvoll erscheint das Kapitel „Einführung in die physikalisch-technischen Grundlagen der Röntgen- und Ultraschalldiagnostik". Beide Techniken sind in der Veterinärmedizin bereits Standardmethoden der bildgebenden Diagnostik, deren Interpretation den Studierenden oder noch nicht so erfahrenen Tierärzten schon frühzeitig zur Kenntnis gebracht werden müssen. Zusätzlich eingefügt wurde ein Kapitel „Einführung in die Computertomographie und CT-Schnittbildanatomie". Die Vermittlung der computertomographisch erfassbaren Normalstruktur schafft eine solide Basis für das Verständnis dieser immer häufiger eingesetzten Untersuchungsmethode. Nicht nur Universitäten, sondern zunehmend auch private, spezialisierte Kleintierpraxen setzen die Computertomographie in der weiterführenden Diagnostik ein. Das letzte, sehr umfangreiche Kapitel (ca. 30 Seiten) beschäftigt sich mit Beiträgen zur klinisch-funktionellen Anatomie. In diesem Teil findet der Leser Informationen zur Arthroskopie und Endoskopie, häufigen organspezifischen Erkrankungen und Abbildungen zu deren Diagnostik unter Verwendung bildgebender Verfahren, wie hoch qualitativer Röntgenaufnahmen, Ultraschallbildern und Computertomogrammen. Zusätzlich finden sich als Erläuterung für den praktischen Bezug zu den anatomischen Sachverhalten auch Zeichnungen und Fotografien. Zusammenfassend berücksichtigt dieses Buch neben den Grundlagen der Anatomie klinisch wichtige Anwendungen und stellt nicht nur für den Studierenden der Veterinärmedizin, sondern auch für praktizierende Tierärzte ein sehr praxisbezogenes Nachschlagewerk dar.

A. Probst