Infection of cattle with Border disease virus by sheep on communal alpine pastures

Braun, Ueli ; Bachofen, Claudia ; Büchi, Romain ; Hässig, Michael ; Peterhans, E

Abstract: The purpose of this study was to investigate whether sheep grazing communal alpine pastures with cattle can transmit Border disease virus (BDV) to cattle. A total of 1170 sheep and 923 cattle were tested for BDV using RT-PCR (sheep) and for pestivirus antibodies using an ELISA (cattle), respectively, before being moved to one of 4 pastures (A, B, C and D). Eight sheep from pasture C were viraemic. 396 of 923 cattle examined before the pasture season were seronegative. The latter were re-examined after the pasture season and 99 were seropositive or indeterminate. Antibody specificity was determined in 25 of these using a serum neutralization test (SNT). BDV infection was confirmed in 10 cattle and was considered likely in 8 others. BVDV infection was confirmed in 4 cattle and considered likely in 3 after pasturing. The study has shown that the transmission of BDV from sheep to cattle is possible on communal alpine pastures.

DOI: https://doi.org/10.1024/0036-7281/a000428

Infection of cattle with Border disease virus by sheep on communal alpine pastures

U. Braun[1], C. Bachofen[2], R. Büchi[1], M. Hässig[1], E. Peterhans[2]

[1]Department of Farm Animals, University of Zurich, [2]Institute of Veterinary Virology, University of Berne

Summary

The purpose of this study was to investigate whether sheep grazing communal alpine pastures with cattle can transmit Border disease virus (BDV) to cattle. A total of 1170 sheep and 923 cattle were tested for BDV using RT-PCR (sheep) and for pestivirus antibodies using an ELISA (cattle), respectively, before being moved to one of 4 pastures (A, B, C and D). Eight sheep from pasture C were viraemic. 396 of 923 cattle examined before the pasture season were seronegative. The latter were re-examined after the pasture season and 99 were seropositive or indeterminate. Antibody specificity was determined in 25 of these using a serum neutralization test (SNT). BDV infection was confirmed in 10 cattle and was considered likely in 8 others. BVDV infection was confirmed in 4 cattle and considered likely in 3 after pasturing. The study has shown that the transmission of BDV from sheep to cattle is possible on communal alpine pastures.

Keywords: sheep, cattle, communal alpine pasture, border disease, bovine viral diarrhoea

Infektion von Rindern durch Schafe auf Alpweiden mit Border-Disease-Virus

In der vorliegenden Arbeit wurde abgeklärt, ob das Border-Disease-Virus (BD-Virus, BDV) während der gemeinsamen Alpung von Schafen und Rindern auf das Rind übertragen werden kann. Dazu wurden die auf vier Alpen (A, B, C, D) zur Sömmerung angemeldeten 1170 Schafe mittels RT-PCR auf BD-Virus und die 923 Rinder serologisch auf Pestivirus-Antikörper untersucht. Bei 8 Schafen der Alp C konnte in den Blutproben BD-Virus nachgewiesen werden. Von 923 untersuchten Rindern waren vor der Alpung 396 Rinder mittels ELISA seronegativ getestet worden. Diese Tiere wurden nach der Alpung erneut untersucht und bei 99 Rindern wurden dabei Pestivirus-Antikörper im Blut nachgewiesen oder der Befund war für eine Infektion sehr verdächtig. Bei 25 dieser Tiere konnte die Spezifität der Antikörper mittels Serumneutralisationstest (SNT) genauer bestimmt werden. Aufgrund der SNT-Untersuchung galt eine Infektion mit dem BD-Virus bei 10 Rindern als sicher und bei 8 Rindern wurde sie vermutet. Im Bezug auf BVDV (Bovine Virusdiarrhoe) wurde eine Infektion bei 4 Rindern nachgewiesen und bei 3 Rindern vermutet. Die Untersuchungen zeigen, dass bei

gemeinsamer Alpung von Schafen und Rindern eine Infektion von Rindern durch Schafe mit dem Border Disease-Virus möglich ist.

Schlüsselwörter: Schaf, Rind, Alpung, Border Disease, Bovine Virusdiarrhoe

Introduction

Pestiviruses occur in many ruminant populations throughout the world. Bovine viral diarrhoea virus (BVD virus, BVDV) affects cattle and Border disease (BD virus, BDV) principally affects sheep. Pestiviruses have the ability of interspecies transmissibility. Recent studies (Krametter-Frötscher et al., 2008; Reichle, 2009; Büchi, 2009) have indicated that BDV can be transmitted from persistently infected sheep to cattle that are co-pastured with sheep. The first report (Cranwell et al., 2007) of cattle infected with BDV was from Great Britain and involved three animals. The first was a 13-month old heifer with diarrhoea and weight loss, the second was a 2.5-year old cow with diarrhoea and other signs of mucosal disease and the third was a small weak newborn calf that died soon after birth. Another recent report (Strong et al., 2010) of 5 cattle infected with BDV was also from Great Britain. Research on the eradication of BVD in Switzerland carried out at the Institute of Veterinary Virology, University of Berne, found several calves persistently infected with BDV. These calves tested positive for pestiviruses in ear punch biopsy samples and subsequently in blood samples and sequencing confirmed persistent infection with BDV, rather than BVDV. Taken together these reports suggest that the presumed natural resistance of cattle against BDV no longer holds true (Strong et al., 2010). Because the seroprevalence of BDV infection of sheep in Switzerland is considerable (20 % in registered flocks and 65 % in large mixed flocks; Schaller et al., 2000), the occurrence of persistently-infected sheep and ongoing transmission of the virus to other sheep must be assumed. Furthermore, it is very likely that in Switzerland sheep will be an important source of pestiviruses for cattle that are pastured or housed with sheep. This is because the program for eradication of BDV, which was started in 2008, will soon be completed. It is suspected that analogous to the spread of BVDV among cattle (Braun et al., 1998) and BDV among sheep and goats (Krametter-Frötscher et al., 2007), communal alpine pasturing plays a role in the transmission of BDV from sheep to cattle. Thus, in addition to generating scientific interest, the transmission of pestiviruses from sheep to cattle has considerable practical relevance. The goal of the present study was to investigate whether BDV is transmitted from sheep to cattle under natural conditions during communal alpine pasturing, and whether infection of seronegative cattle leads to seroconversion during the pasture period.

Animals, Material and Methods

Communal alpine pastures

The study included 4 alpine pastures (A, B, C, D) in the cantons Schwyz, Uri and Obwalden used during the summer of 2008 (Tab. 1). The pastures were 1000 to 2300 m above sea level and varied from 200 to 800 ha (mean, 400 ha). All 4 alpine pastures were grazed by cattle and sheep for 86 to 104 days (mean, 94 days) during the summer.

Sheep

There was a total of 1170 sheep of all ages from 29 private flocks on the 4 pastures. The number of sheep per flock ranged from 7 to 300 sheep (mean, 40.3 sheep) and most were "Weisses Alpenschaf" sheep.

Cattle

There was a total of 923 cattle between 5 months and 13.8 years old (mean ± sd = 29.1 ± 21.18 months) from 94 herds. The majority (n = 825, 89.4 %) were Swiss Braunvieh and the remaining 98 included utility crossbreeds (n = 23), Simmental (n = 23), Limousin (n = 14) and others (n = 18). All tested negative for pestivirus antigen under the ongoing BVD eradication program.

Blood testing of sheep

In all sheep, 6 ml blood was collected from a jugular vein into an evacuated EDTA tube (Vacuette, Greiner Bio-One GmbH, A-Kremsmünster) to test for Border disease antigen before communal pasturing.

Blood testing of cattle

In all cattle, a blood sample was collected from the coccygeal vessels for determination of antibody titre to pestivirus before communal pasturing. From 380 animals that were seronegative, a second blood sample was collected at the end of pasturing to test for seroconversion. Sixteen animals that were seronegative in the first test could not be re-tested because they died or were slaughtered or sold.

Detection of viral RNA in the blood of sheep

This test was carried out at the Institute of Veterinary Virology, University of Berne, using quantitative RT-PCR as recently described (Büchi, 2009).

102

103 ELISA and serum neutralisation

104 Serological testing using ELISA was carried out at the same laboratory (Büchi, 2009). Cattle with a
105 negative ELISA result in the first sample that had a positive or indeterminate result in the second
106 sample underwent a serum neutralisation test (SNT) (Büchi, 2009) to identify the pestivirus for which
107 the animal had seroconverted. Because of genetic similarities between BVDV and BDV, some degree
108 of cross-neutralisation is expected. A BDV titre that was at least twice as high as the BVDV titre
109 confirmed BDV infection, and a titre that was higher than but not twice as high as the BVDV titre
110 indicated a likely BDV infection. The assessment of BVDV titres was analogous.

111

112 Statistical analysis

113 The program StatView 5.1 (SAS Institute, Wangen, Switzerland) was used for statistical evaluation.
114 The means, standard deviations and frequency distributions were calculated for the variables studied
115 and differences were analysed using analysis of variance (ANOVA) and the Bonferroni-Dunn post
116 hoc test. The Wilk Shapiro test was used to test distributions for normality. Results of normally
117 distributed variables are given as mean ± standard deviation and results of variables with a skewed
118 distribution as median and range. The level of significance was set at $P < 0.05$.

119

120 **Results**

121 Virus prevalence in sheep

122 Border disease virus was detected in 8 of 1170 (0.68 %) clinically healthy sheep; 5 had a very high
123 viral load and 3 had a weak positive result. These 8 sheep originated from 2 flocks and all were on
124 pasture C (Tab. 2). The flock prevalence on pasture C was 6.9 %. The other 3 communal pastures
125 were free of BDV-positive sheep. In the 5 sheep with a high viral load, the virus was characterized
126 using sequencing; four viruses belonged to the Swiss BDV subgroup (Reichert, 2009; Peterhans et al.,
127 2010) and the remaining case was BD-3-virus.

128

129 Seroprevalence of cattle before pasturing

130 Of the 923 cattle tested using ELISA, 396 (42.9 %) were seronegative and 527 (57.1 %) were
131 seropositive. The seroprevalence varied from 45.1 to 74.7 % among the four pastures (D 45.1 %, C
132 56.8 %, A 59.4 %, B 74.7 %).

133

134 Seroprevalence of cattle after pasturing

Of the 380 cattle that underwent a second ELISA after pasturing (16 animals were not available for re-testing), 52 had a positive and 47 had an indeterminate result. Of these 99 animals with confirmed or likely seroconversion, 70 were from pasture C, where the BDV-positive sheep were diagnosed. Of the remaining 29 animals, 20, 6 and 3 were from pastures D, A and B, respectively.

Serum neutralisation testing of the 99 samples from cattle with confirmed or likely seroconversion revealed 47 samples in which the antibody could not be differentiated because the BVDV and BDV titres were similar, and 27 samples that were negative (Tab. 3). Differentiation of the antibody was possible in the remaining 25 samples. In 10 samples the BDV titre was at least twice as high as the BVD titre, confirming BDV infection. In 8 samples, the BDV titre was clearly higher than, but not double, the BVD titre and BDV infection was considered likely. In 4 samples the BVDV titre was at least twice as high as the BDV titre, confirming BVDV infection, and in the remaining 3 samples, the BVDV titre was clearly higher than, but not double, the BDV titre and BVDV infection was considered likely. Of the 18 cattle with confirmed or likely BDV infection, 13 were from pasture C and 5 from pasture D. Of the cattle that acquired BVDV infection, 4 were from pasture C and 3 from pasture D.

Discussion

Border disease virus was detected in blood of eight of 1170 (0.68 %) sheep. Five had a high viral load and 3 had a low load; a persistent infection was assumed in the former, but this could not be confirmed because a second blood sample was not collected. The 3 sheep with a low viral load were considered transiently infected, most likely following contact with the persistently-infected sheep. These 8 sheep originated from 2 large commercial flocks, which had had no abortions or births of weak lambs in the past few years. It is noteworthy that the virus-positive sheep were clinically healthy, which was in agreement with several reports of healthy persistently-infected lambs (Barlow et al., 1980; Bonniwell et al., 1987; Nettleton et al., 1992). Likewise, a study of Swiss sheep flocks showed that the majority of BDV infections are subclinical or infected lambs only have mild clinical signs that may go unnoticed. Under such circumstances, a strong economic incentive for a thorough diagnostic workup may be lacking and therefore additional testing is not usually undertaken (Braun et al., 2002). There is little awareness of BD among sheep farmers in Switzerland, although the endemic occurrence of this disease has long been documented (Schaller et al., 2000). Spanish studies reported similar BDV prevalences of 0.3 to 0.6 % (Valdazo-González et al., 2006, 2008) and one of these, a slaughterhouse study, revealed that the prevalence of BDV in individual sheep remained unchanged for several years (Valdazo-González et al., 2008). Five of 21 randomly tested Spanish flocks had BDV-positive sheep, yielding a flock prevalence of 23.8 % (Valdazo-González et al., 2006), which

169 was considerably larger than in the present study (2 of 29 flock, 6.9 %). Most sheep of the present
170 study were from breeding farms, which typically have a lower virus prevalence than commercial
171 flocks because of less animal traffic (Schaller et al., 2002).
172 The seroprevalence of BVDV in cattle before pasturing was 57.1 %, which was comparable to the
173 value of 57.6 % previously observed in Swiss cattle (Rüfenacht et al., 2000); the BVD eradication
174 program, which was started in the same year the present study was carried out, evidently had not yet
175 affected the seroprevalence. Because of the relatively high seroprevalence, only 43 % of cattle could
176 be used to investigate seroconversion, which had occurred in 25 % (99) of the 396 examined cattle.
177 Because of the BVD eradication program, it is projected that the proportion of seropositive cows will
178 approach zero. The risk of seroconversion to pestivirus will therefore increase because the proportion
179 of seronegative cattle will increase, at least theoretically, to 100 %. The relatively large proportion of
180 negative serum neutralisation test results was most likely due to the inclusion of samples with
181 indeterminate ELISA results. Because a BVDV ELISA was used in the present study, its sensitivity
182 for BDV was expected to be somewhat reduced. Indeterminate ELISA results can also be caused by
183 non-specific serum reactions, which generate negative serum neutralisation test results. Based on high
184 titres in the serum neutralisation test, BDV infection was confirmed in 10 cattle and considered likely
185 in 8 others, and the majority of these were from pasture C where the BDV-positive sheep were the
186 most plausible source of infection. Five others were from pasture D, which did not have any BDV-
187 positive sheep, and reasons for seroconversion in cattle included false-negative test results in sheep,
188 transiently viraemic sheep, premature parturition, normal delivery or abortion of persistently-infected
189 lambs and subsequent infection of cattle, infection immediately before the start of co-pasturing and
190 infection from wild ruminants. Wild ruminants were seen on all 4 pastures and contact between the
191 two species was possible. However, the role of wild ruminants in the infection of cattle, sheep and
192 goats remains unclear (Krametter et al., 2004; Vil•ek and Nettleton, 2006; Danuser et al., 2009) and
193 despite high seroprevalences reported from a variety of countries (Lillehaug et al., 2003; Olde
194 Riekerink et al., 2005), transmission of pestivirus to domesticated cattle has not been documented
195 (Vil•ek and Nettleton, 2006). Indirect infection via vectors or fomites are theoretically possible but
196 difficult to substantiate (Houe, 1995).
197 The infection of 7 cattle with BVDV in the absence of any BVDV-positive cattle at the start of the
198 season is noteworthy. Possible reasons for this are generally the same as those used to explain the
199 infection of cattle with BDV on pasture D where there were no known carrier sheep. Because of the
200 BVD eradication program, there should be very few BVDV-infected cows in the future. Whether
201 BDV infection of cattle is clinically relevant and can produce persistently-infected calves needs
202 further study, but recent reports of persistently-infected calves from England (Cranwell et al., 2007)

and Switzerland (personal communication, Institute of Veterinary Virology, University of Berne) suggest that it can. The relationship between BVDV infection of cattle and BDV infection of sheep is reminiscent of the relationship between caprine arthritis-encephalitis (CAE) of goats and Maedi Visna in sheep; CAE virus can be transmitted to sheep, and Maedi-Visna virus of sheep, which is closely related to the CAE-virus, can be transmitted to goats. These relationships complicate serological disease surveillance (Mordasini et al., 2006).

Conclusions

This study has clearly shown that sheep grazing communal alpine pastures with cattle must be considered a risk factor for the transmission of BDV to the latter, at least under Swiss farming conditions. There is no doubt that cattle seropositive for BDV will complicate the BVD eradication program; plans are in place for confirming seronegativity of cattle herds to BVD using milk or blood samples starting as early as 2012. The ELISA test for BVD will be positive in BDV-infected cattle, which will necessitate retesting using a serum neutralisation test.

Acknowledgements

We thank Dr. Josef Risi, cantonal veterinarian of Uri, Schwyz, Obwalden and Unterwalden, for his support, the farmers for their help with sample collection and the staff of the Institute of Veterinary Virology, University of Berne, for laboratory examinations.

References

Barlow, R. M., Vantsis, J. T., Gardiner, A. C., Rennie, J. C., Herring, J. A., Scott, F. M. M.: Mechanisms of natural transmission of border disease. J. Comp. Pathol. 1980, 90: 57-65.

Bonniwell, M. A., Nettleton, P. F., Gardiner, A. C., Barlow, R. M., Gilmour, J. S.: Border disease without nervous signs or fleece changes. Vet. Rec. 1987, 120: 246-249.

Braun, U., Schönmann, M., Ehrensperger, F., Hilbe, M., Brunner, D., Stärk, K. D. C., Giger, T.: Epidemiology of bovine virus diarrhea in cattle on communal alpine pastures in Switzerland. J. Vet. Med. A 1998, 45: 445-452.

Braun, U.,Hilbe, M., Ehrensperger, F., Salis, F., Alther, P., Strasser, M., Stalder, H. P., Peterhans, E.: Border Disease in einem Schafbetrieb. Schweiz. Arch. Tierheilk. 2002, 144: 419-426.

Büchi, R.: Epidemiologische Untersuchungen über die Ansteckung von Rindern durch mit Border-Disease-Virus infizierte Schafe auf Alpweiden. Thesis, University of Zurich, 2009.

Cranwell, M. P., Otter, A., Errington, J., Hogg, R. A., Wakeley, P., Sandvik, T.: Detection of border disease virus in cattle. Vet. Rec. 2007, 161: 211-212.

Danuser, R., Vogt, H. R., Kaufmann, T., Peterhans, E., Zanoni, R.: Seroprevalence and characterization of pestivirus infections in small ruminants and new world camelids in Switzerland. Schweiz. Arch. Tierheilk. 2009, 151: 109-117.

Houe, H.: Epidemiology of bovine viral diarrhea virus. Vet. Clin. North. Am. (Food Anim. Pract.) 1995, 11: 521-547.

Krametter, R., Nielsen, S. S., Loitsch, A., Froetscher, W., Benetka, V., Moestl, K., Baumgartner, W.: Pestivirus exposure in free-living and captive deer in Austria. J. Wildl. Dis. 2004, 40: 791-795.

Krametter-Froetscher, R., Kohler, H., Benetka, V., Moestl, K., Golja, F., Vil•ek, S., Baumgartner, W.: 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 2007, 54: 209-213.

Krametter-Frötscher, R., Benetka, V., Möstl, K., Baumgartner, W.: Transmission of border disease virus from sheep to calves – a possible risk factor for the Austrian BVD eradication programme in cattle? Wien. Tierärztl. Mschr. 2008, 95: 200-203.

Lillehaug, A., Vikøren, T., Larsen, I. L., Åkerstedt, J., Tharaldsen, J., Handeland, K.: Antibodies to ruminant alpha-herpesviruses and pestiviruses in Norwegian cervids. J. Wildl. Dis. 2003, 39: 779-786.

Mordasini, F., Vogt, H. R., Zahno, M. L., Maeschli, A., Nenci, C., Zanoni, R., Peterhans, E., Bertoni, G.: Analysis of the antibody response to an immunodominant epitope of the envelope glycoprotein of a lentivirus and its diagnostic potential. J. Clin. Microbiol. 2006, 44: 981-991.

Nettleton, P. F., Gilmour, J. S., Herring, J. A., Sinclair, J. A.: The production and survival of lambs persistently infected with border disease virus. Comp. Immunol. Microbiol. Infect. Dis. 1992, 15: 179-188.

Olde Riekerink, R. G. M., Dominici, A., Barkema, H. W., De Smit, A. J.: Seroprevalence of pestivirus in four species of alpine wild ungulates in the High Valley of Susa, Italy. Vet. Microbiol. 2005, 108: 297-303.

Peterhans, E., Bachofen, C., Stalder, H., Schweizer, M.: Cytopathic bovine viral diarrhea viruses (BVDV): emerging pestiviruses doomed to extinction. Vet. Res. 2001, 41: 44.

Reichert, C.: Infektion von Kälbern, Schafen und Ziegen mit Border-Disease-Virus. Thesis, University of Zurich, 2009.

Reichle, S. F.: Untersuchungen bei Kälbern, die mit Border-Disease infizierten Lämmern zusammengehalten werden. Thesis, University of Zurich, 2009.

Rüfenacht, J., Schaller, P., Audigé, L., Strasser, M., Peterhans, E.: Prevalence of cattle infected with bovine viral diarrhoea virus in Switzerland. Vet. Rec. 2000, 147: 413-417.

Schaller, P., Vogt, H. R., Strasser, M., Nettleton, P. F., Peterhans, E., Zanoni, R.: Seroprävalenz von Maedi-Visna und Border Disease in der Schweiz. Schweiz. Arch. Tierheilk. 2000, 142: 145-153.

Strong, R., La Rocca, S. A., Ibata, G., Sandvik, T.: Antigenetic and genetic characterisation of border disease virus isolated from UK cattle. Vet. Microbiol. 2010, 141: 208-215.

Valdazo-González, B., Alvarez-Martínez, M., Greiser-Wilke, I.: Genetic typing and prevalence of border disease virus (BDV) in small ruminant flocks in Spain. Vet. Microbiol. 2006, 117: 141-153.

Valdazo-González, B., Alvarez, M., Sandvik, T.: Prevalence of border disease virus in Spanish lambs. Vet. Microbiol. 2008, 128: 269-278.

Vil•ek, S., Nettleton, P. F.: Pestiviruses in wild animals. Vet. Microbiol. 2006, 116: 1-12.

303 Table 1: Description of communal alpine pastures.

Variable	Communal alpine pasture			
	A	B	C	D
Size (ha)	250	200	800	350
Elevation above sea level (metres)	1000 – 1900	1500 – 2000	1200 – 2300	1400 – 2200
Length of pasture season (days)	95	104	90	86
Number of sheep	126	70	671	303
Number of flock owners	2	5	11	10
Number of cattle	165	91	514	153
Number of cattle owners	12	7	51	23

304

305

306 Table 2: Assessment of 1170 sheep for the presence of border disease virus in blood using RT-PCR

Pasture	A	B	C	D
No of sheep: Viremic/total	0/126	0/70	8/671	0/303

307

308

309 Table 3: Assessment of 99 positive or indeterminate serum neutralisation test results (cattle).

Assessment of SNT results	Communal pasture				Total
	A	B	C	D	
Antibody not differentiated	3	0	36	8	47
SNT negative	3	3	17	4	27
BDV infection confirmed	0	0	7	3	10
BDV infection likely	0	0	6	2	8
BVDV infection confirmed	0	0	2	2	4
BVDV infection likely	0	0	2	1	3

310

311 SNT, Serum neutralisation test

312 BDV, Border disease virus

313 BVDV, Bovine viral diarrhoea virus

314