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Specificity of pestivirus antibodies in wild ruminants from Switzerland

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Introduction

Switzerland started a bovine viral diarrhoea (BVD) national eradication campaign in 2008. BVD virus (BVDV) belongs together with classical swine fever virus (CSFV) of pigs and border disease virus (BDV) of sheep to the genus *Pestivirus*. Ruminant pestiviruses are not strictly host-specific and interspecies virus transmission is possible (Vilček and Nettleton, 2006). In Switzerland, the shared use of Alpine pastures by multiple cattle herds during the summer grazing season is a widespread tradition. In this practice, cattle can share the grazing grounds with wild ruminants; these may be recipients as well as donors of pestiviruses. In the Pyrenees, for example, massive losses in chamois due to infection with border disease virus are reported (Arnal et al., 2004). In the French and Italian Alps, pestivirus antibodies have been detected in chamois, ibex, roe and red deer, mouflons and wild boar (Olde Riekerink et al., 2005; Fernandez et al., 2011). In order to determine if wild ruminants may play a role in pestivirus epidemiology in Switzerland, a virological and serological survey was carried out in 2009–2011 (Casaubon et al., 2012). Results revealed very low seroprevalences in Alpine chamois (*Rupicapra rupicapra rupicapra*), Alpine ibex (*Capra ibex ibex*) and red deer (*Cervus elaphus elaphus*) of 2.1 %, 1.8 % and 2.7 %, respectively. The determination of the specificity of pestivirus antibodies, i. e. against which pestivirus species they are directed, can give indication on the origin of the infection and therefore enhance our knowledge on interspecies transmissions of pestiviruses to wild ruminants. Therefore, the present study was raised with the aim to specify the antibodies detected in red deer, Alpine chamois and ibex by using a comparative serum neutralization test (SNT).

Material and Methods

Sera from 9 Alpine chamois, 4 ibexes and 4 red deer were included in the present study. All animals were hunted in 2009 in Switzerland, namely in the cantons of Wallis, St. Gallen, Glarus, Nidwalden, Graubünden, Appenzell and Obwalden. The analyzed sera originated from a previous study performed by Casaubon et al. (2012) and were selected according to two criteria: the sera tested positive in an inhouse BVDV antibody detection ELISA and enough volume of serum was left to perform the SNT. The SNT was done using 5 different pestivirus strains: 2 BVDV strains, one of type 1 and 2 each, and three BDV strains. The BVDV-1h virus 04-01b was isolated in 2004 at the Institute of Veterinary Virology from serum of a Swiss PI cow. The BVDV-2a strain CS8644 was kindly provided by G. Wolf (Institute of Medical Microbiology, Infectious and Epidemic Diseases, Munich, Germany). The BDV-Swiss strain CH-BD4 originates from leukocytes of a Swiss PI sheep and belongs to a subgenotype of BD viruses preliminary named "BDV-Swiss". The BDV-3 virus used was the first BDV isolated in Switzerland. It originates from a PI sheep and is described as CH-BD1 (Stalder et al., 2005). The BDV-4 strain H2121/1 was isolated from tissue of a Pyrenean chamois from Andorra (Arnal et al., 2004) and was kindly provided by Peter Nettleton (Moredun Research Institute, Edinburgh, Scotland). While BVDV-1h, BDV-Swiss and BDV-3 strains are widespread in Switzerland, BVDV-2 and BDV-4 have never been isolated in this country. SNT was performed using primary bovine turbinate cells for the BVD viruses and primary lamb synovial membrane cells for the BD viruses as previously described (Bachofen et al., 2008). Since the volumes of sera available were very limited, sera were pre-diluted 1:16 in Earle's minimal essential medium followed by serial 2-fold dilutions over 12 steps. After the virus was added, the final serum dilutions ranged from 1:32 to 1:65'536. Each serum-dilution and virus mixture was distributed over 8 wells of a microtitre plate, pre-seeded with bovine and ovine cells. The serum neutralization titre was expressed as the reciprocal of the serum dilution capable to inhibit infection in 50 % of the wells. For being able to calculate even low titres, we made the assumption that if any well of the 1:32 dilution was positive, the underlying 1:8 and 1:16 dilutions were positive, too. Titres between 23.8 (one single well positive in the 1:32 dilution) and 45.3 thus represent only the highest possible titre and may in reality be lower but not negative. Titres were calculated according to the method of Reed and Muench (1938). A titre difference of 3-fold or more was considered as a significant difference and hence decisive for an infection by the pestivirus species yielding the highest titre as recommended by the OIE guideline (OIE, 2008).

Results

Neutralizing antibody titres against the different pestivirus strains are shown in Table 1.

The more than 3-fold higher titre of chamois no. 1 against the BVDV-1h strain is congruent with detection of viral RNA of the same BVDV-1 subgroup in this chamois kid (Casaubon et al., 2012). However, most of the chamois (no. 2, 3, 4, 5 and 6) had higher titres (> 2 fold) against the BDV strains, which points to sheep being the source of infection rather than cattle. Chamois no. 2, 3 and 7 showed significantly (> 3 fold) higher titres against BDV-3 and BDV-4 than against BDV Swiss. However, the differences between BDV-3 and -4 titres were too small to clearly determine the specificity within the BDV strains. Since BDV-4 has never been detected in Switzerland, it is likely that these animals had contact to BDV-3 viruses. Chamois no. 4, 5 and 6 had similarly high titres against BDV-3, BDV-4 and BDV-Swiss strains. In chamois no. 8 and 9, titres were generally very low and no meaningful differences between the titres against BDV and BVDV were detected.

The ibex no. 1 and 2 showed significantly higher titres against BDV than against the two BVDV strains. Ibex no. 3 had generally low titres and no clear antibody determination was possible. The titres of ibex no. 4 were very similar for all 5 viruses but not noticeably low.

The antibody titres in red deer were generally low and thus a clear determination of the antibody specificity was difficult. However, of the 4 tested deer, 3 (no. 1, 3 and 4) had higher antibody titres against the BVDV strains, and in deer no. 3 and 4, titres against BVDV-1h were significantly higher, suggesting that they had been infected with this virus strain. In only one case (red deer no. 2) the titres were similarly high against BVDV and BDV.

Discussion

According to the OIE guideline, a titre difference of 3-fold or more should be considered decisive for an infection by the pestivirus species yielding the highest titre (OIE, 2008). However, when comparing different subgroups of the same pestivirus species, this rule may be too stringent. Serological cross-reactivity between different pestiviruses has to be taken in consideration when interpreting our results. The extent of cross-reactivity depends on the strain of ruminant pestivirus involved and the interval between infection and time of sampling (Wensvoort et al., 1989). It is interesting to note that there seems to be a rather high cross-reactivity between the BDV viruses from Switzerland, particularly the BDV-3 strain and BDV-4, which was associated to severe outbreaks of disease in chamois in the Pyrenees (Arnal et al., 2004). The finding of low titre antibodies against the BVDV-2a strain in chamois no. 9 should be interpreted with caution. Infection with this strain has been occasionally reported in Europe but have so far not been detected in Switzerland.

Although the sample size is low, our results provide a first indication that Swiss chamois are more likely to be infect-

Table 1: Neutralizing antibody titres of chamois, ibex and red deer sera against five pestivirus strains. Titres between 23.8 (one single well positive in the 1:32 dilution) and 45.3 represent only the highest possible titre and may in reality be lower but not negative. Titres < 23.8 may be negative (no well positive with lowest dilution being 1:32).

	BVDV-1h	BVDV-2a	BDV-Swiss	BDV-3	BDV-4
chamois 1	14000	406	891	3440	955
chamois 2	689	448	446	1450	3170
chamois 3	80.6	< 23.8	190	589	1150
chamois 4	448	556	2720	2430	2900
chamois 5	294	119	549	1024	1450
chamois 6	156	< 23.8	588	1280	838
chamois 7	90.5	< 23.8	36.8	446	362
chamois 8	25.4	32	< 23.8	25.4	< 23.8
chamois 9	< 23.8	40.3	< 23.8	< 23.8	< 23.8
ibex 1	340	156	1570	1540	2500
ibex 2	105	223	1024	771	955
ibex 3	< 23.8	< 23.8	64	36.8	40.3
ibex 4	401	119	223	680	588
red deer 1	32	25.4	< 23.8	< 23.8	< 23.8
red deer 2	137	55.7	105	55.7	50.8
red deer 3	236	27.9	32	23.8	< 23.8
red deer 4	85	< 23.8	25.4	< 23.8	< 23.8

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ed with BDV than BVDV, while deer are usually infected with BVDV. This finding might be due to chamois and deer displaying differences in susceptibility to the different pestiviruses. More likely, however, the difference in natural habitat determines which pestivirus species wild animals are most likely to become infected with. On summer pastures, sheep and goats are grazing often on higher and steeper pastures than cattle and are more likely to come in contact to chamois and ibex. Indeed, a previous study showed that contacts between Alpine chamois and sheep are frequently occurring (Ryser-Degiorgis et al., 2002) and in Switzerland, pestivirus infection in sheep are usually caused by BDV (Danuser et al., 2009). Also in case of ibex, contacts to cattle have been recorded but interactions with sheep seem to be more common (Casaubon et al., 2012), which may explain the presence of BDV specific antibodies in these animals. Red deer live at lower altitudes than chamois and ibex and they are more likely to have contact with BVDV infected cattle all year round, not only in the summer pastures but also during winter around feeding places. Interestingly, in contrast to sheep, where BDV-3 and BDV-Swiss are found with similar frequency (unpublished data), chamois may not be infected very frequently by BDV-Swiss viruses. In nearly all cases investigated here, the titres were higher against BDV-3 and BDV-4 than against BDV-Swiss. However, data of the present study indicate a high cross-reactivity between the three BDV strains used, which suggest a high degree of antigenic similarity between these strains. Alternatively, the similarity in titres may be due to the antibodies being directed against virus of a yet unidentified BDV subgroup that was, obviously, not included in the SNT but may be circulating in wild ruminants in Switzerland.

In conclusion, Alpine chamois, Alpine ibex and red deer populations in Switzerland can be infected with different pestivirus species. Even if not posing a risk for the eradication of BVDV, contact between cattle and wild ruminants infected with pestivirus could interfere with the serological surveillance during the BVDV eradication program. Furthermore, our results suggest that yet unidentified pestiviruses could be circulating in wild ruminants in Switzerland. Thus, further investigations on pestiviruses in non-bovine virus hosts are indicated.

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