Seroprevalence and characterization of pestivirus infections in small ruminants and new world camelids in Switzerland

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Summary

The seroprevalence of pestivirus infections in small ruminants and new world camelids in Switzerland was determined. In 5'059 sera of sheep from 382 herds, 503 sera of goats from 54 herds and 109 sera of alpacas and lamas from 53 herds, population prevalences of 16.1 % (sheep), 25.4 % (goats) and 4.6 % (new world camelids), respectively, were found. In order to determine the source of infection, the serological reactions were further characterized by cross-neutralization against two pestiviruses representing the genotypes BVDV (Bovine Virus Diarrhea Virus)-1 and BDV (Border Disease Virus)-1. Based on the ratio of respective antibody titres, 56.1 % of the infections in sheep were induced by a BDV-1, 12.9 % by a BVDV-1 and 31.0 % by an unresolved pestivirus. In goats, the corresponding proportions were 23.4 %, 10.2 % and 66.4 %, respectively. In Alpacas and Lamas, the source of infection of 1 animal was BDV-1 and that of 4 seropositive animals remained unresolved. In view of the phylogenetic relationship between pestiviruses, the unresolved source of infection is most probably attributable to other pestivirus genotypes circulating in small ruminants and new world camelids. Due to the predominance of pestiviral genotypes other than BVDV-1, the risk of transmission of BVDV from persistently infected small ruminants and new world camelids to cattle appears to be moderate, apart from close direct contact in mixed animal husbandry, communal pasturing and grazing in the alps.

Keywords: Bovine Virus Diarrhea Virus, Border Disease Virus, sheep, goat, cross-species transmission

Seroävelänsel und Charakterisierung von Pestivirusinfektionen bei Kleinwiederkäuern und Neuweltkameliden in der Schweiz


Schlüsselwörter: Bovines Virusdiarrhoe-Virus, Border Disease Virus, Schaf, Ziege, Zwischenarten-Transmission
Introduction

Pestiviruses are economically important infectious agents of even-toed ungulates (order artiodactyla), causing considerable losses notably in cattle and swine (Ruefenacht et al., 2001; Fourichon et al., 2005). As pathogens of domestic animals, at least six genetically defined species (genotypes) within the genus pestivirus belonging to the Flaviviridae family are recognized to date, consisting of Border Disease Virus 1–3 (BVDV-1,2,3), Bovine Viral Diarrhoea Virus types 1 and 2 (BVDV-1,2) and the Classical Swine Fever Virus (CSFV) (Riekerink et al., 2005; Stalder et al., 2005; Vlcek und Nettleton, 2006). Despite high genetic variability there is a strong antigenic relatedness of all species or genotypes resulting in a high serological cross-reactivity within the genus (Dekker et al., 1995; Konig et al., 2003; Becher et al., 2003). Pestiviruses can cross the species barrier, whereby adaptation to different host species including cattle, sheep, swine or wildlife ruminants could be the origin of the phylogenetic divergence within the group (Uttenthal et al., 2005).

An estimated 70–90 % of pestiviral infections occur without overt clinical disease (Ames, 1986), however, infection may decrease the production of milk or meat. Due to its immunosuppressive effect, an acute pestivirus infection is an important factor contributing to enteric or respiratory disease in young animals in feedlots (Steck et al., 1980; Weiss et al., 1994). More dramatically, it can cause hemorrhagia (mostly BVDV-2) in all age categories or, in pregnant animals, abortion, persistent infection and/or malformation of the foetus (Moennig und Liess, 1993; Goens, 2002). Epidemiologically, the most important feature of pestiviral infections is the ability to generate persistently infected (PI) offspring when the first infection of the mother occurs within a certain time frame of pregnancy (40–120 days in cattle, 20–80 days in sheep; Barlow, 1990; Moennig und Liess, 1993). By shedding large amounts of virus throughout their lives, PI animals represent the main source of infection for susceptible hosts. Upon specific genomic mutations/recombination events or upon superinfection with a cytopathic pestiviral strain, PI cattle develop mucosal disease and eventually die within few days. Sheep persistently infected with BVDV may exhibit the so-called “hairy shaker syndrome”, which is characterized by abnormal fleece growth and muscle tremors in lambs, which may die later of a mucosal disease-like condition.

The seroprevalence of BVDV in Swiss cattle ranges from 60–80 % and the prevalence of PI animals was estimated to be approximately 1 % (Ruefenacht et al., 2000; Stalder et al., 2005). The seroprevalence of Border disease Virus was estimated at 20 % (Schaller et al., 2000) whereas the prevalence of BDV PI animals is currently not known. The aim of this project was to determine the current seroprevalence of pestivirus infections in small ruminants, alpacas and lamas and to differentiate serologically between BDV and BVDV as a source of the infection. This is important in view of the BVDV eradication program in Swiss cattle, since pestivirus infection in other ruminants could potentially act as a source of reinfection, jeopardizing the aims of the eradication project.

Animals, Material and Methods

Animals

Five thousand and fifty-nine blood samples of sheep aged more than 12 months were collected in 382 herds within the framework of the official random sample for the surveillance of Brucellosis in 2006. Depending on herd size (hs), all animals (hs <40), 40 animals (hs 40 – 99) or 50 animals (hs ≥ 100), respectively, were sampled. Five hundred and three samples of goats aged more than 6 months from 54 herds were selected from the official random sample for the surveillance of Caprine Arthritis Encephalitis virus (CAEV) in 2005. All of these samples were kindly provided by the laboratories involved in the diagnostics of notifiable animal epidemics. Furthermore, 109 blood samples of Alpacas (n = 77) and Lamas (n = 32) were collected from the Clinic for Ruminants in Bern, in 2006/07. Sera obtained after centrifugation of the blood samples at 1’400g were kept at -20°C until serological testing.

Detection of BVDV and BDV antibodies

An ELISA was used as an initial screening test for the selection of positive samples and performed as previously described (Canal et al., 1998). Briefly, ELISA microtitre trays (Maxisorp, A/S Nunc, Kamstrup, Denmark) were coated with BVD viral antigen derived from cell cultures infected with the cytopathic strain R1935/72 (Oregon C24V, subgenotype BVDV-1a). Sera diluted 1:10 were then added to the coat, which favors the binding of antibodies directed to the well-conserved non-structural NS23 protein. As a conjugate, Protein-G-Peroxidase (Bioreba AG, Basel, Switzerland) was used. All samples with positive reaction were then confirmed by serum-neutralization test (SNT), using both BVDV-1a R1935/72 and BDV-1 Moredun reference strain (Barlow, 1972) as challenge viruses.

SNT was performed as described (Steck et al., 1980). Briefly, heat inactivated sera (56°C for 30 min) were diluted in Earles-MEM (Earle’s minimal essential medium, Flow Laboratories, Allschwil, Switzerland) using two-fold dilutions (1:4 to 1:512), and incubated at equal volume with a virus stock solution containing 100 TCID50 (50 % tissue culture infective dose) for 60 min at 37°C and 5 % CO2. For each dilution, four wells of microtitre trays (TPP, Trasadingen, Switzerland) containing an approximately 80 % confluent monolayer of BVDV-/BDV-free secondary foetal bovine turbinate cells were then inoculated (100 ul/well). Subsequently, microtitre trays were incubated for 5 days at 37°C and 5 % CO2 and then evaluated directly.
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for a cytopathic effect (cp BVDV-1) or after immunoperoxidase staining in case of the noncytopathic BDV (Thür et al., 1997). The resulting titres were calculated according to Spearman-Kaerber (Spearman, 1908; Kaerber, 1931) by extrapolating the dilution of the sample reducing the number of virus positive wells to 50%. Titre values equal to or greater than 1:5 were considered positive. Titres that were at least four times higher in one of the two assays were taken to be significantly different (Office International des Epizooties, 2004), thus indicative for the corresponding viral type being the source of infection. Therefore, by using the ratio of the BVDV-1 SNT titre and the BDV-1 SNT titre (Q = BVDV-1/BDV-1), Q values ≥ 4 indicated BVDV-1, such as 0.25 BDV-1 as source of infection, whereas intermediate ratios (0.25 < Q < 4) pointed toward an unresolved source of infection.

Prevalence and herd prevalence

The population prevalence (Pp) was defined as the proportion of seropositive animals in a given group of animals. For the herd prevalence (Ph), herds with at least one seropositive animal were considered to be positive.

Results

Sheep

Positive results by ELISA, which were confirmed by SNT, were considered to be true positive results and used for the calculation of the prevalence (irrespective of Q). Of the 5059 sheep sera tested we found 826 to be positive by ELISA. Of these, 704 were positive by BVDV-1 SNT (p = 13.9%), 748 (p = 14.8%) by BDV-1 SNT and 815 (p = 16.1%) by either pestivirus SNT. Overall, samples of 382 herds were tested, 110 of which were positive for BVDV-1 or BDV-1 (28.8%), resulting in an overall herd prevalence of 30.1%.

According to the canton of origin, marked differences in both population (ranging from 0% to 35.4% for BVDV-1 and 0% to 33.7% for BDV-1 SNT, respectively) and herd prevalences (7.5% to 75.0% for BVDV-1 and 7.5 to 65.6 for BDV-1 SNT, respectively) were seen. The highest population prevalences were found in the cantons of Glarus, Grison, Ticino and St. Gallen (Tab. 1). The within-herd prevalences in herds with seropositive animals ranged from 2.5% to 100% with an average of 35% (data not shown). Due to incomplete sampling of the herds, these results were not further evaluated. Sensitivity and specificity of ELISA as compared to SNT were at 0.96 and 0.97, respectively.

For positive sera, the ratio of the two SNT titres (Q) were used to determine the seroconversion-inducing pestivirus as BVDV-1, BDV-1 or as an unresolved pestivirus. According to this definition, the source of infection of 457 animals (56.1%) was classified as BDV-1, that of 105 animals (12.9%) as BVDV-1 and that of 253 animals (31.0%) as an unresolved pestivirus (Tab. 3). For the herd status, the titre ratios (Q) of all seropositive animals were averaged, using the same ranges for classification as above. Usually, the Q values of single animals within a herd were quite homogeneous (not shown), indicating rather single than multiple infection sources. Apart from the differences in seroprevalence (Tab. 1), the geographical distribution of the herds at the time of sampling according to their serological status did not exhibit particular clustering with regard to the pestiviral genotypes (Fig. 1).

Figure 1: Geographical distribution of sheep herds according to their serological status defined by average Q (SNT, ratio of BVDV-1 and BDV-1 titres).
Table 1: Prevalence of BVDV-1 and BDV-1 neutralizing antibodies in blood samples of sheep aged over 12 months collected in 382 herds within the framework of the official random sample for the surveillance of Brucellosis in 2006.

<table>
<thead>
<tr>
<th>Canton</th>
<th>n: total samples</th>
<th>BVDV-1 SNT pos (%)</th>
<th>BDV-1 SNT pos (%)</th>
<th>either SNT pos (%)</th>
<th>n: total herds</th>
<th>BVDV-1 SNT pos (%)</th>
<th>BDV-1 SNT pos (%)</th>
<th>either SNT pos (%)</th>
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<td>50 (13.7)</td>
<td>32</td>
<td>5 (15.6)</td>
<td>5 (15.6)</td>
<td>5 (15.6)</td>
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<tr>
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<td>8 (20.0)</td>
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<td>2 (-)</td>
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<td>7 (-)</td>
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<td>0 (-)</td>
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<td>5 (2.8)</td>
<td>5 (2.8)</td>
<td>19</td>
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<td>2 (-)</td>
<td>2 (-)</td>
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<td>GE</td>
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<td>1 (-)</td>
<td>1 (-)</td>
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<td>3 (-)</td>
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<td>60 (22.7)</td>
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<td>6 (27.3)</td>
<td>6 (27.3)</td>
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<td>8 (21.1)</td>
<td>8 (21.1)</td>
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<td>1 (-)</td>
<td>1 (-)</td>
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<td>2 (-)</td>
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<td>0 (-)</td>
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<td>OW</td>
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<td>1 (-)</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>1</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>SG</td>
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<td>100 (25.4)</td>
<td>104 (26.3)</td>
<td>29</td>
<td>16 (-)</td>
<td>16 (-)</td>
<td>16 (-)</td>
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<td>1 (2.7)</td>
<td>0 (0)</td>
<td>1 (2.7)</td>
<td>2</td>
<td>1 (-)</td>
<td>0 (-)</td>
<td>1 (-)</td>
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<tr>
<td>SG</td>
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<td>0 (-)</td>
<td>0 (-)</td>
<td>1</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
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<td>TG</td>
<td>229</td>
<td>17 (7.4)</td>
<td>17 (7.4)</td>
<td>17 (7.4)</td>
<td>16</td>
<td>5 (-)</td>
<td>5 (-)</td>
<td>5 (-)</td>
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<tr>
<td>TI</td>
<td>201</td>
<td>55 (27.4)</td>
<td>22 (10.9)</td>
<td>57 (28.4)</td>
<td>13</td>
<td>5 (-)</td>
<td>5 (-)</td>
<td>5 (-)</td>
</tr>
<tr>
<td>TR</td>
<td>60</td>
<td>1 (1.7)</td>
<td>1 (1.7)</td>
<td>1 (1.7)</td>
<td>2</td>
<td>1 (-)</td>
<td>1 (-)</td>
<td>1 (-)</td>
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<tr>
<td>UD</td>
<td>82</td>
<td>8 (9.8)</td>
<td>12 (14.6)</td>
<td>12 (14.6)</td>
<td>13</td>
<td>4 (-)</td>
<td>4 (-)</td>
<td>4 (-)</td>
</tr>
<tr>
<td>VS</td>
<td>585</td>
<td>48 (8.2)</td>
<td>59 (10.1)</td>
<td>60 (10.3)</td>
<td>31</td>
<td>5 (16.1)</td>
<td>7 (22.6)</td>
<td>7 (22.6)</td>
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<tr>
<td>ZH</td>
<td>50</td>
<td>6 (1.2)</td>
<td>9 (1.8)</td>
<td>9 (1.8)</td>
<td>40</td>
<td>3 (7.5)</td>
<td>3 (7.5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Total</td>
<td>5059</td>
<td>704 (13.9)</td>
<td>748 (14.8)</td>
<td>815 (16.1)</td>
<td>382</td>
<td>110 (28.8)</td>
<td>110 (28.8)</td>
<td>115 (30.1)</td>
</tr>
</tbody>
</table>

* A herd with at least one seropositive animal is considered to be positive.

Abbreviations used: p = prevalence (calculated only for n ≥ 30)

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Table 2: Prevalence of BVDV-1 and BDV-1 neutralizing antibodies in samples of goats older than 6 months selected from the official random sample for the surveillance of Caprine Arthritis Encephalitis virus (CAEV) in 2005.

<table>
<thead>
<tr>
<th>Canton</th>
<th>n: total samples</th>
<th>BVDV-1 SNT pos (%)</th>
<th>BDV-1 SNT pos (%)</th>
<th>either SNT pos (%)</th>
<th>n: total herds</th>
<th>BVDV-1 SNT pos (%)</th>
<th>BDV-1 SNT pos (%)</th>
<th>either SNT pos (%)</th>
</tr>
</thead>
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<tr>
<td>BE</td>
<td>360</td>
<td>69 (19.2)</td>
<td>69 (19.2)</td>
<td>69 (19.2)</td>
<td>40</td>
<td>6 (-)</td>
<td>6 (-)</td>
<td>6 (-)</td>
</tr>
<tr>
<td>GR</td>
<td>103</td>
<td>45 (43.7)</td>
<td>42 (40.8)</td>
<td>48 (46.6)</td>
<td>8</td>
<td>6 (-)</td>
<td>6 (-)</td>
<td>6 (-)</td>
</tr>
<tr>
<td>TG</td>
<td>22</td>
<td>9 (-)</td>
<td>9 (-)</td>
<td>9 (-)</td>
<td>4</td>
<td>2 (-)</td>
<td>2 (-)</td>
<td>2 (-)</td>
</tr>
<tr>
<td>TI</td>
<td>18</td>
<td>2 (-)</td>
<td>2 (-)</td>
<td>2 (-)</td>
<td>2</td>
<td>1 (-)</td>
<td>1 (-)</td>
<td>1 (-)</td>
</tr>
<tr>
<td>Total</td>
<td>503</td>
<td>123 (24.5)</td>
<td>122 (24.5)</td>
<td>122 (24.5)</td>
<td>54</td>
<td>22 (41.7)</td>
<td>20 (38.5)</td>
<td>20 (38.5)</td>
</tr>
</tbody>
</table>

* A herd with at least one seropositive animal is considered to be positive.

Abbreviations used: p = prevalence (calculated only for n ≥ 30)
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Table 3: Source of infection in seropositive animals and overall seroprevalences.

<table>
<thead>
<tr>
<th>Species</th>
<th>BVDV-1</th>
<th>BDV-1</th>
<th>Pestivirus</th>
<th>Total pos/n (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>105 (12.9)</td>
<td>457 (56.1)</td>
<td>253 (31.0)</td>
<td>815/5059 (16.1)</td>
</tr>
<tr>
<td>Goat</td>
<td>13 (10.2)</td>
<td>30 (23.4)</td>
<td>85 (66.4)</td>
<td>128/503 (25.4)</td>
</tr>
<tr>
<td>Alpaca &amp; Lama</td>
<td>0 (-)</td>
<td>1 (-)</td>
<td>4 (-)</td>
<td>5/109 (4.6)</td>
</tr>
</tbody>
</table>

Abbreviations used: n = number of sera tested, p = overall seroprevalence

Goats

From a total number of 503 serum samples taken from 54 herds located in the cantons of Berne, Grison, Thurgau and Ticino, a total of 123 samples (24.5 %) and 27 herds (50 %) were positive by BVDV-1 SNT. In BDV-1 SNT, 122 sera (24.3 %) and 28 herds (51.9 %) were positive, resulting in overall population and herd prevalences of 25.4 % and 51.9 %, respectively (Tab. 2). The within-herd prevalences in herds with seropositive animals ranged from 3.7 % to 100 % with an average of 43 % (data not shown). Due to incomplete sampling of the herds, these results were not further evaluated. Sensitivity and specificity of ELISA for goat sera as compared to SNT were at 0.98 and 0.98, respectively.

According to the titre ratios (Q), the source of infection of 30 animals (23.4 %) was classified as BDV-1, that of 13 animals (10.2 %) as BVDV-1 and that of 85 animals (66.4 %) as an unresolved pestivirus (Tab. 3). The geographical distribution of the herds and serologic status are shown in Figure 2.

Alpacas and Lamas

Of the 109 sera sampled from 53 herds, 4 tested positive in ELISA and BVDV-1 SNT (3.7 % population- and 7.5 % herd prevalence, respectively). In the SNT with BDV-1 five samples tested positive (4.6 % population- and 9.4 % herd prevalence, respectively). According to the titre ratios (Q), the source of infection of 1 animal was classified as BDV-1 and that of 4 animals as unresolved pestivirus (Tab. 3). All sera from alpacas and lamas were negative in real-time RT PCR (Kaiser, 2001; Marti, 2003; Stalder et al., 2005) for the detection of BVD virus (data not shown), indicating low prevalence of persistently infected animals as the major source of interspecies transmission.

The geographical distribution of the herds of new world camelids is shown in Figure 3.

Discussion

Pestivirus infections in small ruminants are widespread worldwide with a wide range of seroprevalences depending on the management of animal husbandry (Barlow, 1990; Loken et al., 1991; Tabbaa et al., 1995; Nettleton und Enrican, 1995; Zaghawa, 1998; Tegtmeier et al., 2000; Graham et al., 2001; Celedon et al., 2001; Celedon et al., 2001; O’Neill et al., 2004; Berriatua et al., 2004; Krametter-Froetscher et al., 2006; Okur-Gumusova et al., 2006). On Swiss sheep breeding farms, a seroprevalence of 20 % was found in a representative sample of sera collected in 1993 (Schaller et al., 1998).
ELISA was used for the initial screening of sera for the presence of pestivirus antibodies due to its ability to detect a wide spectrum of pestiviruses, since it is predominantly based on the highly conserved and cross-reactive NS3 antigen (Canal et al., 1998; Sandvik, 2007). Using an atypical BVDV-1 strain (subgenotype 1a) not circulating in Swiss cattle for the SNT might result in a lowered estimate for the proportion of BVDV-1 infections, since much higher titres can be observed in a given serum when a pestiviral strain, which is closely related to the infecting strain, is applied for neutralization (Botton et al., 1998; Becher et al., 2003). On the other hand, the strain used was considered to be suitable for the differentiation between BVDV and CSFV due to its particular antigenic properties (Neukirch et al., 1980).

In view of the predominance of other pestiviral genotypes than BVDV-1, the risk of transmission of BVDV from persistently infected small ruminants and new world camelids to cattle appears to be moderate. The introduction of PI animals in sheep by cattle persistently infected with BVDV followed by back-transmission to and possible PI induction in cattle has been shown (Paton et al., 1992; Paton et al., 1997). By contrast, the transmission of BDV from persistently infected small ruminants is less documented and appears to be less likely (Carlsson und Belak, 1994; Nettleton und Entrican, 1995; Krametter-Froetscher et al., 2005). Whateover, due to the probable association of BVDV infections found in sheep with management practices like mixed animal husbandry, communal pasturing and grazing in the alps, these risk factors must be kept in mind for a successful BVDV eradication campaign in cattle in Switzerland.
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