

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 lammas 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

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

In dieser Arbeit wurde die Seroprävalenz von Pestivirusinfektionen bei Kleinwiederkäuern und bei Neuweltkameliden in der Schweiz bestimmt. In 5'059 Seren von Schafen aus 382 Herden, 503 Seren von Ziegen aus 54 Herden und 109 Seren von Alpakas und Lamas aus 53 Herden wurden Prävalenzen von 16.1 % (Schafe), 25.4 % (Ziegen) und 4.6 % (Neuweltkameliden) gefunden. Mittels Kreuz-Neutralisation gegen zwei Pestiviren des Genotyps BVDV (Bovine Virus Diarrhea Virus)-1 und BDV (Border Disease Virus)-1 wurde aufgrund der gefundenen Titerquotienten die Infektionsquelle eruiert. Bei den Schafen waren demnach 56.1 % der Infektionen auf ein Pestivirus des Genotyps BDV-1, 12.9 % auf eines des Genotyps BVDV-1 und 31.0 % auf ein nicht näher bestimmtes Pestivirus eines andern Genotyps zurückzuführen. In Ziegen betrug die entsprechenden Anteile 23.4 %, 10.2 % und 66.4 %. Bei den Neuweltkameliden konnte die Infektionsquelle bei einem von 5 seropositiven Tieren dem Genotyp BDV-1 zugeordnet werden, bei 4 Tieren handelte es sich um nicht näher bestimmte Genotypen. Angesichts der phylogenetischen Verwandtschaft zwischen den Pestiviren handelt es sich bei den nicht näher bestimmten Pestiviren sehr wahrscheinlich um Vertreter aus andern Pestivirus-Genotypen, die bei Kleinwiederkäuern und Neuweltkameliden zirkulieren. Aufgrund des Überwiegens anderer Genotypen als BVDV-1 erscheint das Risiko der Rück-Übertragung von BVDV von persistent infizierten Kleinwiederkäuern und Neuweltkameliden auf Rinder eher gering, abgesehen von engem Kontakt in gemischten Herden oder bei gemeinsamer Weide und Alping.

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 (BDV-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; Vilcek 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; König et al., 2003; Becher et al., 2003). Pestiviruses can cross the species barrier, whereby adaptation to different host species including cattle, goats, 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/ recombinational events or upon superinfection with a cytopathic pestiviral strain, PI cattle develop mucosal disease and eventually die within few days. Sheep persistently infected with BDV 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 Earle's-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

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 ($Q = \text{BVDV-1}/\text{BDV-1}$), 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 (P_p) was defined as the proportion of seropositive animals in a given group of animals. For the herd prevalence (P_h), 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 (≥ 4 or ≤ 0.25) 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).

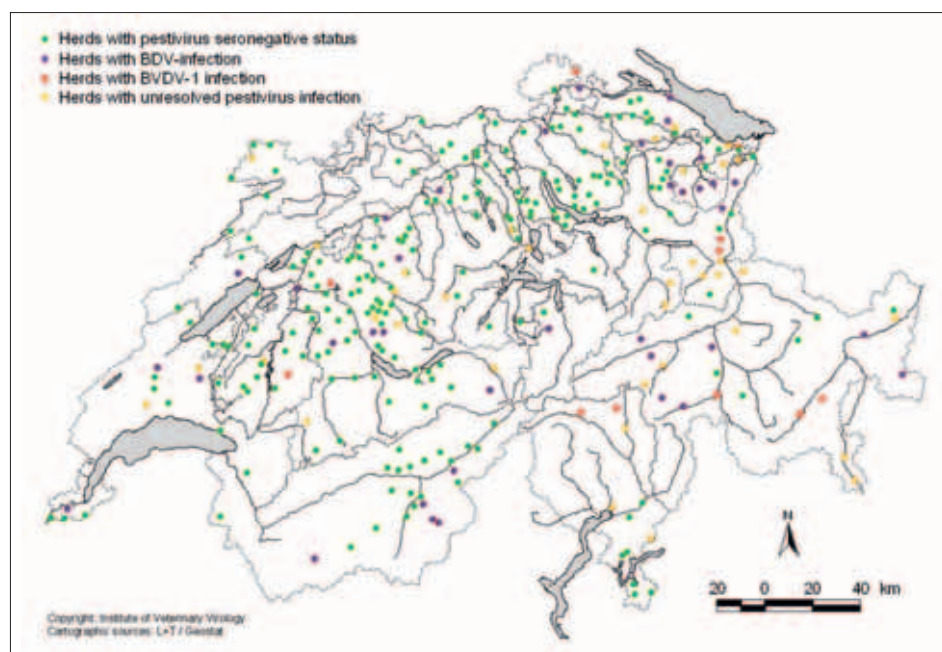


Figure 1: Geographical distribution of sheep herds according to their serological status defined by average Q (SNT, ratio of BVDV-1 and BDV-1 titres).

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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.

Canton	Population				Herds *			
	n: total samples	BVDV-1 SNT pos (%)	BDV-1 SNT pos (%)	either SNT pos (%)	n: total herds	BVDV-1 SNT pos (%)	BDV-1 SNT pos (%)	either SNT pos (%)
AG	366	32 (8.7)	50 (13.7)	50 (13.7)	32	5 (15.6)	5 (15.6)	5 (15.6)
AI	45	8 (17.8)	8 (17.8)	8 (17.8)	2	2 (-)	2 (-)	2 (-)
AR	157	26 (16.6)	27 (17.2)	27 (17.2)	11	7 (-)	7 (-)	7 (-)
BE	1079	104 (9.6)	123 (11.5)	124 (11.5)	111	22 (19.8)	24 (21.6)	24 (21.6)
BL	36	0 (0)	0 (0)	0 (0)	2	0 (-)	0 (-)	0 (-)
FL	39	0 (0)	0 (0)	0 (0)	1	0 (-)	0 (-)	0 (-)
FR	180	5 (2.8)	5 (2.8)	5 (2.8)	19	2 (-)	2 (-)	2 (-)
GE	52	4 (7.7)	5 (9.6)	5 (9.6)	4	1 (-)	1 (-)	1 (-)
GL	48	17 (35.4)	14 (29.2)	17 (35.4)	4	3 (-)	3 (-)	3 (-)
GR	821	266 (32.4)	277 (33.7)	300 (36.5)	32	24 (75.0)	21 (65.6)	24 (75.0)
JU	38	8 (21.1)	8 (21.1)	8 (21.1)	7	1 (-)	1 (-)	1 (-)
LU	27	2 (-)	4 (-)	4 (-)	5	2 (-)	2 (-)	2 (-)
NE	22	3 (-)	6 (-)	6 (-)	3	1 (-)	1 (-)	1 (-)
NW	6	0 (-)	0 (-)	0 (-)	1	0 (-)	0 (-)	0 (-)
OW	6	0 (-)	0 (-)	0 (-)	1	0 (-)	0 (-)	0 (-)
SG	396	93 (23.5)	100 (25.4)	104 (26.3)	29	16 (-)	16 (-)	16 (-)
SH	37	1 (2.7)	0 (0)	1 (2.7)	2	1 (-)	0 (-)	1 (-)
SZ	40	0 (0)	0 (0)	0 (0)	1	0 (-)	0 (-)	0 (-)
TG	229	17 (7.4)	17 (7.4)	17 (7.4)	16	5 (-)	5 (-)	6 (-)
TI	201	55 (27.4)	22 (10.9)	57 (28.4)	13	5 (-)	5 (-)	5 (-)
UR	60	1 (1.7)	1 (1.7)	1 (1.7)	2	1 (-)	1 (-)	1 (-)
VD	82	8 (9.8)	12 (14.6)	12 (14.6)	13	4 (-)	4 (-)	4 (-)
VS	585	48 (8.2)	59 (10.1)	60 (10.3)	31	5 (16.1)	7 (22.6)	7 (22.6)
ZH	507	6 (1.2)	9 (1.8)	9 (1.8)	40	3 (7.5)	3 (7.5)	3 (7.5)
	5059	704 (13.9)	748 (14.8)	815 (16.1)	382	110 (28.8)	110 (28.8)	115 (30.1)

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

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

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.

Canton	Population				Herds *			
	n: total samples	BVDV-1 SNT pos (%)	BDV-1 SNT pos (%)	either SNT pos (%)	n: total herds	BVDV-1 SNT pos (%)	BDV-1 SNT pos (%)	either SNT pos (%)
BE	360	67 (18.6)	69 (19.2)	69 (19.2)	40	18 (45)	19 (47.5)	19 (47.5)
GR	103	45 (43.7)	42 (40.8)	48 (46.6)	8	6 (-)	6 (-)	6 (-)
TG	22	9 (-)	9 (-)	9 (-)	4	2 (-)	2 (-)	2 (-)
TI	18	2 (-)	2 (-)	2 (-)	2	1 (-)	1 (-)	1 (-)
Total	503	123 (24.5)	122 (24.3)	128 (25.4)	54	27 (50)	28 (51.9)	28 (51.9)

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

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

Table 3: Source of infection in seropositive animals and overall seroprevalences.

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

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 lammas 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 Entrican, 1995; Zaghawa, 1998; Tegtmeier et al., 2000; Graham 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

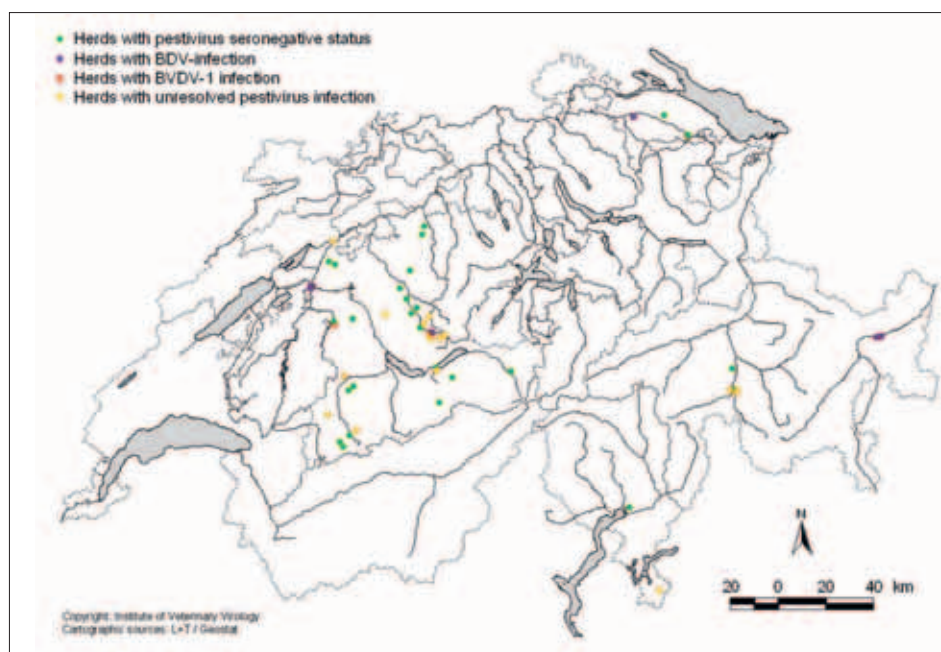


Figure 2: Geographical distribution of goat herds according to their serological status defined by average Q (SNT titre ratio).

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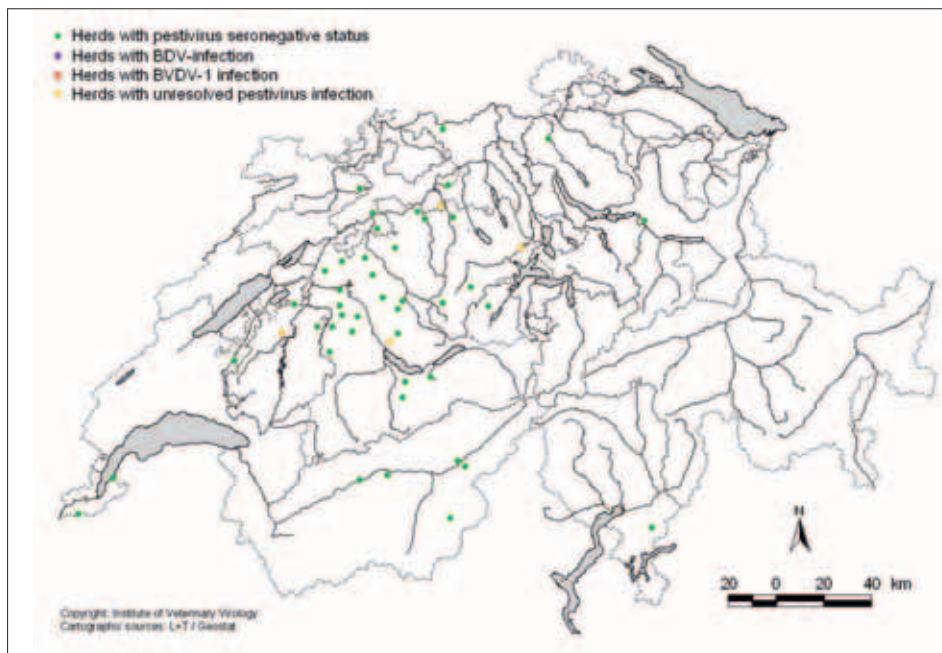


Figure 3: Geographical distribution of Swiss alpaca and lama herds according to their serological status defined by average Q (SNT titre ratio).

et al., 2000). In the present study, a slightly lower seroprevalence of 16.1% was found for sheep, pointing to a rather stable epidemic situation with some regional differences. Higher regional seroprevalences were also associated with a higher prevalence of positive herds, pointing to transmission between herds. The seroprevalences for goats and new world camelids, which had not been determined previously, were at 25.4% and 4.6%, respectively. When considering the widespread practice of communal grazing in the alps of small ruminants with cattle, the type of pestivirus infection circulating in small ruminants and the possibility of interspecies transmission is of particular interest. In sheep, the differential serology based on cross-neutralisation against a classical Border disease- (BDV-1) and a Bovine Virus Diarrhea Virus (BVDV-1) revealed that the majority of infections (56%) were attributable to a virus of the BDV-1 genotype, whereas only 13% of infections may have been induced by a virus of the BVDV-1 genotype. Interestingly, 31% were unresolved, i.e. seemed to be induced by pestiviruses equidistant to the laboratory challenge viruses applied. In view of the current knowledge on pestivirus genotypes, including at least 3 different BDV genotypes (BDV-1-3; Vilcek et al., 1999; Braun et al., 2002; Stalder et al., 2005; Valdazo-Gonzalez et al., 2007), the most likely explanation for this result is the circulation of other pestivirus genotypes in the Swiss sheep population (Stalder et al., 2005). In goats, only 25% of the infections were attributable to BDV-1, whereas 10% and 65% could be classified as BVDV-1 or unresolved pestivirus, respectively, clearly pointing to a different spectrum of pestiviruses circulating in this small ruminant species.

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 crossreactive 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 induction 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). Whatsoever, 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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Séro-prévalence et caractérisation des infections à pestivirus chez les petits ruminants et les camélidés du Nouveau Monde en Suisse

Dans ce travail, on a déterminé la sero-prévalence des infections à pestivirus chez les petits ruminants et les camélidés du Nouveau Monde en Suisse. On a trouvé sur 5'059 sérums de moutons provenant de 382 troupeaux, 503 sérums de chèvres de 54 troupeaux et 109 sérums d'alpacas et lamas de 53 troupeaux, une prévalence de 16.1 % (moutons) 25.4 % (chèvres) et 4.6 % (camélidés) au moyen d'une neutralisation croisée face à deux pestivirus du génotype BVDV (Bovine Virus Diarrhea Virus) – 1 et BDV (Border Disease Virus) – 1 et on a identifié l'origine de l'infection sur la base des quotients de titres trouvés. Chez les moutons, 56.1 % des infections étaient dues à un pestivirus du génotype BDV – 1, 12.9 % au génotype BVDV-1 et 31 % à un pestivirus d'un autre type non déterminé plus précisément. Chez les chèvres, ces proportions étaient de 23.4 %, 10.2 % et 66.4 %. Chez les camélidés du Nouveau Monde, la source de l'infection était chez un des cinq animaux positifs le génotype BDV-1. Chez les 4 autres, il s'agissait de génotypes non déterminés plus précisément. Vu la parenté phylogénétique entre les pestivirus, il s'agit très vraisemblablement, chez les pestivirus non typés plus précisément, de représentants d'autres génotypes de pestivirus qui circulent chez les petits ruminants et les camélidés. Au vu de la prédominance des autres types par rapport au BVDV-1, le risque d'une réinfection de bovins par des BVDV provenant de petits ruminants ou de camélidés infectés permanents semble faible sauf en cas d'un contact étroit dans des troupeaux mixtes ou lors du pâturage ou de l'alpage en commun.

Sieroprevalenza e caratteristiche dell'infezione da Pestivirus nei piccoli ruminanti e nei camelidi del nuovo mondo in Svizzera

In questo studio è stata studiata nei piccoli ruminanti e nei camelidi del nuovo mondo in Svizzera la sieroprevalenza di un'infezione da Pestivirus. Sono stati esaminati i sieri di 5'059 pecore provenienti da 382 mandrie, di 503 capre provenienti da 54 mandrie e di 109 alpaca e lama provenienti da 53 mandrie e è stata constatata una prevalenza del 16.1% (pecore), 25.4% (capre) e 4.6% (camelidi del nuovo mondo). Con l'aiuto di una neutralizzazione incrociata contro due Pestivirus del genotipo BVDV (Bovine Virus Diarrhea Virus)-1 e BDV (Border Disease Virus)-1 si è potuta trovare con la titolazione la fonte dell'infezione. Nelle pecore si trovavano tracce per il 56.1% dell'infezione del Pestivirus di genotipo BDV-1, il 12.9% in uno di genotipo BVDV-1 e il 31.0% da un Pestivirus non definito di un altro genotipo. Nelle capre la percentuale era di rispettivamente 23.4 %, 10.2 % et 66.4 %. Nei camelidi del nuovo mondo si è potuta riscontrare in 1 su 5 animali sieropositivi il genotipo BDV-1. Negli altri 4 animali non si poteva definire il genotipo. Sotto l'aspetto della parentela filogenetica tra i Pestivirus si può affermare che nei casi dove non si è potuto definire un genotipo si tratta probabilmente di un subentrante da altri tipi di genotipo del Pestivirus che circolano tra i piccoli ruminanti e i camelidi del nuovo mondo. A causa dell'importanza che hanno gli altri genotipi che il BVDV-1 sembra alquanto debole il rischio di una retro-trasmissione di BVDV da ruminanti e camelidi del nuovo mondo con infezione persistente a bovini a prescindere dagli stretti contatti nelle mandrie miste o nei pascoli e negli alpeggi.

References

Ames T.R.: The causative agent of BVD: its epidemiology and pathogenesis. *Vet.Med.* 1986, 81: 848–869.

Barlow R.M.: Experiments in border disease. IV. Pathological changes in ewes. *J.Comp. Pathol.* 1972, 82: 151–157.

Barlow, R. M.: Border disease virus. In: *Virus Infections of Ruminants*. Hrsg. Dinter, Z. and Morein, B., Elsevier Science

Publishers B.V., Amsterdam, Oxford, New York, Tokyo, 1990, 267–278

Becher P., Ramirez R.A., Orlich M., Rosales S.C., König M., Schweizer M., Stalder H., Schirrmeyer H., Thiel H.J.: Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. *Virology* 2003, 311: 96–104.

Berriatua E., Barandika J., Aduriz G., Atxaerandio R., Garrido J., Garcia-Perez A.L.: Age-specific seroprevalence of Border dis-

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- ease virus and presence of persistently infected sheep in Basque dairy-sheep flocks. *Vet.J.* 2004, 168: 336–342.
- Botton S.A., daSilva A.M., Brum M.C.S., Weiblen R., Flores E.F.*: Antigenic characterization of Brazilian bovine viral diarrhoea virus isolates by monoclonal antibodies and cross-neutralization. *Brazilian J.Med.Biol.Res.* 1998, 31: 1429–1438.
- Braun U., Hilbe M., Ehrensperger F., Salis F., Alther P., Strasser M., Stalder H.P., Peterhans E.*: Border disease in a flock of sheep. *Schweiz.Arch.Tierheilk.* 2002, 144: 419–426.
- Canal C.W., Strasser M., Hertig C., Masuda A., Peterhans E.*: Detection of antibodies to bovine viral diarrhoea virus (BVDV) and characterization of genomes of BVDV from Brazil. *Vet.Microbiol.* 1998, 63: 85–97.
- Carlsson U., Belak K.*: Border disease virus transmitted to sheep and cattle by a persistently infected ewe – epidemiology and control. *Acta Vet.Scand.* 1994, 35: 79–88.
- Celedon M., Sandoval A., Droguett J., Calfio R., Ascencio L., Pizarro J., Navarro C.*: Survey for antibodies to pestivirus and herpesvirus in sheep, goats, alpacas (*Lama pacos*), llamas (*Lama glama*), guanacos (*Lama guanicoe*) and vicuna (*Vicugna vicugna*) from Chile. *Arch.Med.Vet.* 2001, 33: 165–172.
- Dekker A., Wensvoort G., Terpstra C.*: Six antigenic groups within the genus pestivirus as identified by cross neutralization assays. *Vet.Microbiol.* 1995, 47: 317–329.
- Fourichon C., Beaudeau F., Bareille N., Seegers H.*: Quantification of economic losses consecutive to infection of a dairy herd with bovine viral diarrhoea virus. *Prev.Vet.Med.* 2005, 72: 177–181.
- Goens S.D.*: The evolution of bovine viral diarrhoea: a review. *Can.Vet.J.-Rev.Vet.Can.* 2002, 43: 946–954.
- Graham D.A., Calvert V., German A., McCullough S.J.*: Pestiviral infections in sheep and pigs in Northern Ireland. *Vet.Rec.* 2001, 148: 69–72.
- Kaerber G.*: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Arch.exp.Pathol.Pharmakol.* 1931, 162: 480–487.
- Kaiser D*: Entwicklung und Evaluation eines RT-TaqMan-PCR Testverfahrens zum Nachweis des BVD-Virus. Dissertation, Universität Bern, 2001.
- Konig M., Rosales S.C., Becher P., Thiel H.J.*: Heterogeneity of ruminant pestiviruses: Academic interest or important basis for the development of vaccines and diagnostics? *Berl.Munch.Tierarztl.Wschr.* 2003, 116: 216–221.
- Krametter-Froetscher R., Loitsch A., Kohler H., Schleiner A., Schiefer P., Moestl K., Golja F., Baumgartner W.*: Prevalence of antibodies to pestiviruses in goats in Austria. *J.Vet.Med.B Infect. Dis.Vet.Public Health* 2006, 53: 48–50.
- Krametter-Froetscher R., Loitsch A., Mostl K., Sommerfeld-Stur I., Baumgartner W.*: Seroprevalence of border disease and bovine viral diarrhoea in sheep and goats in selected regions of Austria. *Wien.Tierarztl.Monatsschr.* 2005, 92: 238–244.
- Loken T., Krogsrud J., Larsen I.L.*: Pestivirus infections in Norway – serological investigations in cattle, sheep and pigs. *Acta Vet. Scand.* 1991, 32: 27–34.
- Marti S.*: Nachweis der BVD-Virusinfektion in Einzel- und Tankmilchproben mittels real-time-PCR. Dissertation, Universität Bern, 2003.
- Moennig, V. and Liess, B.*: Pathogenesis of intrauterine infections. In: Proceedings of the second symposium of pestiviruses. Hrsg. Edwards, S., Fondation Marcel Mérieux, Lyon, 1993, 91–97
- Nettleton P.F., Entrican G.*: Ruminant pestiviruses. *Br.Vet.J.* 1995, 151: 615–642.
- Neukirch M., Liess B., Frey H.R., Prager D.*: Serologische Beziehungen zwischen Stämmen des Europäischen Schweinepestvirus und dem Virus der Bovinen Virusdiarrhoe. *Fortschr.Vet. Med.* 1980, 30: 148–153.
- O'Neill R.G., O'Connor M., O'Reilly P.J.*: A survey of antibodies to pestivirus in sheep in the Republic of Ireland. *Irish Vet.J.* 2004, 57: 525–530.
- Office International des Epizooties*: Equine Rhinopneumonitis. In: Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals. Hrsg. Blancou, J. and Trusczyński, M., Office International des Epizootie, Paris, 2004, 1–17.
- Okur-Gumusova S., Yazici Z., Albayrak H.*: Pestivirus seroprevalence in sheep populations from inland and coastal zones or Turkey. *Rev.Méd.Vét.* 2006, 157: 593–596.
- Paton D., Gunn M., Sands J., Yapp F., Drew T., Vilcek S., Edwards S.*: Establishment of serial persistent infections with bovine viral diarrhoea virus in cattle and sheep and changes in epitope expression related to host species. *Arch.Virol.* 1997, 142: 929–938.
- Paton D.J., Simpson V., Done S.H.*: Infection of pigs and cattle with bovine viral diarrhoea virus on a farm in England. *Vet.Rec.* 1992, 131: 185–188.
- Riekerink R.G.M.O., 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.
- Ruefenacht J., Schaller P., Audige L., Knutti B., Kupfer U., Peterhans E.*: The effect of infection with bovine viral diarrhoea virus

on the fertility of Swiss dairy cattle. *Theriogenology* 2001, 56: 199–210.

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

Sandvik T.: Bovine viral diarrhoea diagnostics – Established facts and recent developments. *Cattle Pract.* 2007, 15: 178–183.

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.

Spearman C.: The method of “right or wrong cases” (constant stimuli) without Gauss’s formulae. *Brit.J.Psychol.* 1908, 2: 227–242.

Stalder H.P., Meier P., Pfaffen G., Wageck-Canal C., Ruefenacht J., Schaller P., Bachofen C., Marti S., Vogt H.R., Peterhans E.: Genetic heterogeneity of pestiviruses of ruminants in Switzerland. *Prev.Vet.Med.* 2005, 72: 37–41.

Steck F., Lazary S., Fey H., Wandeler A., Huggler C., Oppliger G., Baumberger H., Kaderli R., Martig J.: Immune responsiveness in cattle fatally affected by bovine virus diarrhoea-mucosal disease. *Zbl.Vet.Med.B* 1980, 27: 429–445.

Tabbaa D., Giangaspero M., Nishikawa H.: Seroepidemiological survey of Border disease (BD) in Syrian Awassi sheep. *Small Ruminant Res.* 1995, 15: 273–277.

Tegtmeier C., Stryhn H., Uttenthal A., Kjeldsen A.M., Nielsen T.K.: Seroprevalence of border disease in Danish sheep and goat herds. *Acta Vet.Scand.* 2000, 41: 339–344.

Thür B., Hilbe M., Strasser M., Ehrensperger F.: Immunohistochemical diagnosis of pestivirus infection associated with bovine and ovine abortion and perinatal death. *Am.J.Vet.Res.* 1997, 58: 1371–1375.

Uttenthal A., Grondahl C., Hoyer M.J., Houe H., van Maanen C., Rasmussen T.B., Larsen L.E.: Persistent BVDV infection in mouseteers infects calves – Do we know the reservoirs for BVDV? *Prev.Vet.Med.* 2005, 72: 87–91.

Valdazo-Gonzalez B., Alvarez-Martinez M., Sandvik T.: Genetic and antigenic typing of border disease virus isolates in sheep from the Iberian Peninsula. *Vet.J.* 2007, 174: 316–324.

Vilcek S., Nettleton P.F.: Pestiviruses in wild animals. *Vet.Microbiol.* 2006, 116: 1–12.

Vilcek S., Paton D., Lowings P., Bjorklund H., Nettleton P., Belak S.: Genetic analysis of pestiviruses at the 3′ end of the genome. *Virus Genes* 1999, 18: 107–114.

Weiss M., Hertig C., Strasser M., Vogt H.-R., Peterhans E.: Bovine Virusdiarrhoe/Mucosal Disease: Eine Übersicht. *Schweiz.Arch.Tierheilk.* 1994, 136: 173–185.

Zaghawa A.: Prevalence of antibodies to bovine viral diarrhoea virus and/or Border disease virus in domestic ruminants. *J.Vet.Med.B Infect.Dis.Vet.Public Health* 1998, 45: 345–351.

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