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New World camelids and Bovine Virus Diarrhea Virus (BVDV) infection in Switzerland

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New World camelids (NWC) are becoming more and more popular. In 2009 2'652 llamas and 2'094 alpacas were registered in Swiss farms (Bundesamt für Statistik, Berne CH). A wide range of viral infections are known in NWC, like West Nile Virus, contagious ecthyma and Bovine virus diarrhea virus. Persistent BVDV (Bovine Virus Diarrhea Virus) infections have been reported in recent years in alpacas (Goyal et al., 2002; Mattson et al., 2006; Foster et al., 2005 and 2007; Carman et al., 2005; Barnett et al., 2008; Byers et al., 2009; Kim et al., 2009) and in llamas (Belknap et al., 2000; Wentz et al., 2003).

BVDV belongs to the genus pestivirus within the flaviviridae family. The genus consists of the two genetic species BVDV-1 and -2, which are again divided into numerous subgroups. Among BVD viruses, cytopathogenic (cp) and non-cytopathogenic (ncp) types are distinguished due to their properties when cultured on cells. Only the non-cytopathogenic type causes persistent infection in cattle when affecting fetuses during the immunotolerant phase of gestation (Bachofen et al., 2008; Van Amstel and Kennedy, 2010). In alpacas a ncp BVDV genotype 1b was isolated (Goyal et al., 2002; Carman et al., 2005; Byers et al., 2009; Kim et al., 2009) and in llamas also a BVD type 1b was recognized (Wentz et al., 2003). A serological survey among 63 alpaca herds all over the USA found 16 herds (25,4 %) harboring seropositive and 4 (6,3 %) with persistent infected (PI) offsprings (Topliff et al., 2009). A similar survey in Switzerland including 53 camelid herds with 109 sera examined detected a seroprevalence of 4,6 % (Danuser et al, 2009). A newer serological survey of 596 serum samples showed that the prevalence of BVDV carriers was 0 % (Mudry et al., 2010).

Attempts were made to provoke the birth of PI offsprings by experimental infection of 4 pregnant llamas between days 65 and 105 of gestation. But neither were clinical signs observed in the mothers nor were PI offsprings born (Wentz et al., 2003). Because the gestation period in NWC is longer than in cattle (alpacas: range 335 to 356 days; NWC mean 345 days), the period of susceptibility of the camelid fetus for a persistent infection has not been determined with certainty (Carman et al., 2005; Mattson et al. 2006; Byers et al., 2009; Kapil et al., 2009; Van Amstel and Kennedy, 2010). Supposed that the ontogenesis of the immune system is similar to bovids, Mattson et al. (2006) postulate the development of a PI offspring until approximately 145 days of gestation as possible. Other authors (Byers et al., 2009) propose that the gestational exposure time for BVDV immunotolerance in alpacas may be only during the first trimester. In a newer study, transplacental infection during early gestation of alpacas naturally exposed to BVDV type 1b was confirmed in 7 out 10 live-born offsprings (Bedenice et al., 2011). In PI offsprings clinical symptoms like anorexia, decreased weight gain, chronic recurrent debilitation and infections as well as diarrhea can be found and some show congenital defects. Stillbirths and abortions are also seen in affected herds (Evermann, 2006; Foster et al., 2007; Byers et al., 2009; Passler and Walz, 2009; Topliff et al., 2009; Van Amstel and Kennedy, 2010; Bedenice et al., 2011). To diagnose BVDV in NWC the same diagnostic tests as for cattle can be used, like immunohistochemistry, antigen detection ELISA, PCR or virus isolation (Carman et al., 2005; Kapil et al., 2009). By using immunohistochemistry large amounts of antigen can be found in PI offsprings in several organs (Byers et al., 2009). As source of infection in NWC movement of animals i.e. for mating is suspected. Another possibility would be the contact to infected cattle, mixed animal husbandry and communal pastures (Evermann, 2006; Foster et al., 2007; Barnett et al., 2008; Danuser et al., 2009; Passler and Walz, 2009; Topliff et al., 2009; Van Amstel and Kennedy, 2010).

Because an eradication of BVDV is in progress in Switzerland, the aim of this study was to exclude infected NWC as a possible source of reinfection of bovines by identifying BVDV infected animals. We therefore used retrospectively paraffin-embedded material from 94 alpacas, 59 llamas, 5 guanacos and 8 vicuñas referred for necropsy to the Institute of Veterinary Pathology, between 1996 and 2009 and serum samples from NWC collected in 2007 to identify possible infection with pestiviruses, mainly BVDV. Most animals were from the German part of Switzerland (mostly from the canton of Zurich, Fig. 1) and the gender and age distribution is summarized in Table 1. In addition to this retrospective data, 99 serum samples collected from sound animals for another study (Kaufmann

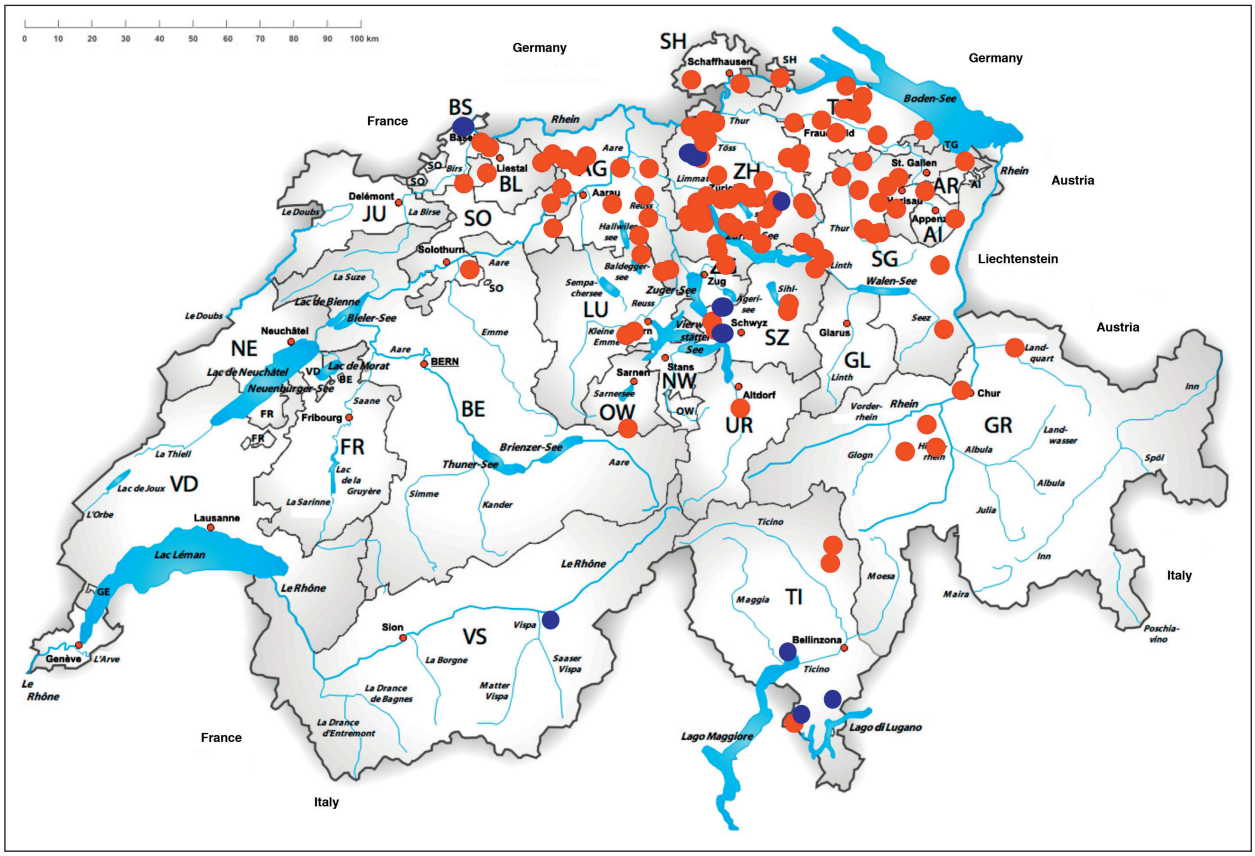

Figure 1: Distribution of the New World camelids camelids (NWC) examined. Blue dots is the localization of serum sampling, red dots is the localization of NWC from where animals were received for necropsy.

Table 1: Age and gender distribution of cases analyzed by immunohistochemistry.

Species	Male	Female	Abortion	Minimum age (years)	Maximum age (years)
Alpaca	36	52	8	neonatal	27
Llama	27	27	5	neonatal	16
Guanaco	1	3	1	neonatal	12
Vicuña	1	5	2	neonatal	23

et al., 2010; Zanolari et al., 2010) were analyzed by ELISA for BVD antigen. Serum samples originated from 77 alpacas and 24 llamas. Age and gender distribution of these samples is presented in Table 2.

Tissue sections mostly of brain but also skin, spleen, kidney, liver and/or gastrointestinal tract were used for immunohistochemistry and performed as described previously by Hilbe et al. (2007). As a positive control the analogous stained brain section from a PI calf was used. The HerdChek*BVDV Ag/Serum Plus detects BVDV antigens in serum, plasma, whole blood and ear notch tissue samples. Specific monoclonal antibodies (Erns/gp 44–48) are coated on the microtiter plates, so that captured BVD-antigens can be detected (HerdChek* BVDV Antigen ELISA Ear-Notch/Serum Test Kit; Idexx Laboratories). This ELISA is not established in NWC; however, it was used in previous studies or mentioned in reviews (Foster et al., 2005; Kapil et al., 2009; Van Amstel and Kennedy, 2010).

Table 2: Number of serum samples and age distribution in Alpacas and Llamas for BVD antigen ELISA, age and gender distribution.

Species	Male	Female	Minimum age (years)	Maximum age (years)
Alpaca	20	57	0.1	20
Llama	9	15	0.1	18.5

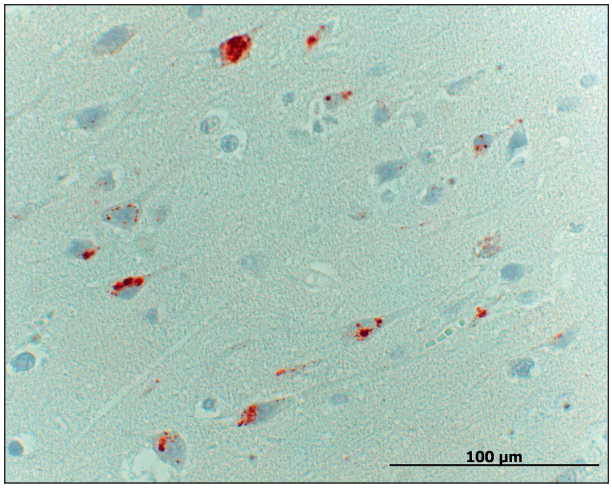

Figure 2: Bovine brain, BVDV positive control: Immunohistochemistry, EnVision-method, 40x. Note the intracytoplasmic red labelling of neurons.

Results

Tissues of 166 animals were examined for BVDV antigen by means of immunohistochemistry as described above. In no animal, BVDV antigen could be detected by this test (Fig. 2). Two out of the 101 sera examined were repeatedly antigen positive. A skin biopsy of these cases was requested. For immunohistochemistry, however, only one case could be examined and revealed to be negative.

Discussion

Organs from 166 animals and 101 sera were tested for BVDV antigen. Assuming that the population number of NWC in Switzerland was around 4'000 at the time of sampling (Bundesamt für Statistik, Berne, Switzerland), approximately 6,7 % of this population was involved in our investigation. Danuser et al. (2009) found a seroprevalence of 4,9 %. Mudry et al. (2010) found an overall pestivirus antibody seroprevalence of 5,75 % and a prevalence of 0 % for pestiviral RNA in the year 2008 in NWC in Switzerland, showing that the infection rate is low. Still, movement of animals for mating or contact to infected cattle as well as mixed animal husbandry and communal pastures have to be regarded as a source of infection in NWC (Foster et al., 2007; Barnett et al., 2008; Topliff et al., 2009; Danuser et al., 2009).
Immunohistochemistry is described in the literature as a strong tool for identifying PI in NWC (Carman et al., 2005; Byers et al., 2009). With the HerdChek*BVDV Ag/Serum Plus we had 2 positive samples out of 101 sera. One of the positive serum was confirmed to be false positive by IHC. Kapil et al. (2009) described this phenomenon and they postulated that commercial antigen-capture ELISA can cause false positive results because of high background. The antigen-ELISA has not been validated for camelids (Van Amstel and Kennedy, 2010). Unfortunately, the second positive serum sample could not be rechecked by IHC testing because the owner of the animal was not willing to carry out further examinations. The PI NWC described until now in the literature were infected mostly by the genotype 1b. In Switzerland the viral genetics of 169 Swiss isolates from bovines confirmed the presence of the BVDV-1 subgroups b, e, h and k. No BVDV type 2 was detected in this study (Bachofen et al., 2008). Most animals harbored the subgroup BVDV-1e, followed by 1h, 1k and 1b. The subgroup BVDV-1b was found in less than 10 % of the cases (Bachofen et al., 2008). Therefore, assuming that NWC are more prone for a persistent infection with the subgroup 1b it can be hypothesized that the infection rate of NWC is lower than in other countries because of a lower circulating level of this type in ruminants in Switzerland.

Conclusion

In Switzerland an infection of NWC with the subgroup BVDV-1, mostly with the genotype 1b is not very likely. Therefore, the occurrence of PI offsprings in NWC as a virus source can be regarded as a rare event and NWC a minor thread to the eradication efforts for BVD in Switzerland.

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