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SHORT COMMUNICATION

Bovine virus diarrhea and the vector-borne diseases Anaplasmosis and Bluetongue: a sero-surveillance in free-ranging red deer (*Cervus elaphus*) in selected areas of Switzerland

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Abstract Due to climate changes, diseases emerging from southerly adjacent areas (Mediterranean countries) are likely to spread northward. Expanded migration of red deer harbors the risk of introducing new pathogens into a naive population of either wild or domestic animals. Little is known about the importance of red deer as a potential reservoir for diseases of domestic ruminants in Switzerland. Deer is susceptible for all three agents that were selected in this study: bovine virus diarrhea virus (BVDV), *Anaplasma marginale* (AM), and Bluetongue virus (BTV). The goal of this project was to establish the serological status of red deer in Switzerland concerning BVDV, AM, and BTV, and to assess the possible impact of disease dynamics with a focus on potential transmission of these diseases from red deer to cattle or vice versa. Sampling areas were selected according the following criteria: abundance of red deer, potential insect vector distribution due to climatic conditions, and traditional alpine pasture husbandry along with known migration routes of red deer. Blood samples were collected during the regular hunting season 2004 and 2005 by hunters and gamekeepers. There was no serological evidence for the presence of the vector-borne diseases AM and BT in red deer in Switzerland. Four out of 234 sera showed a positive result for BVD, corresponding to a sero-prevalence of 1.7% (95% CI 0.46–4.38). Facing the fact of the high sero-prevalence for BVD in Swiss cattle (60–80%) disease transmission from red deer to cattle in these areas under investigation is rather unlikely.

Keywords Sero-surveillance · Red deer · Bovine viral diarrhea · Vector-borne diseases · Bluetongue · Anaplasmosis · Switzerland

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Introduction

In mountain areas of Switzerland, red deer and domestic ruminants share overlapping habitats and are likely to be exposed to the same infectious diseases. Interactions between wild and domestic ruminants occur and are sometimes observed (Hauser, personal communication). Moreover, red deer usually migrate from their winter habitats located in the southern part of Switzerland and the southerly adjacent Italy or Austria to their summer habitats in the higher Alps. This seasonal migration has to be considered as a risk factor for introduction of new pathogens into a naive population, either red deer or domestic ruminants.

The two vector-borne diseases Anaplasmosis (AM) and Bluetongue (BT) claim increasing attention as emerging

diseases because there is evidence for the occurrence of these infectious agents in the ruminant population in southern Europe (Caracappa et al. 2003; De La Fuente et al. 2004). Due to climate changes and intensified international animal trade, these vector-borne diseases may also reach areas previously free of such infections (Mellor and Wittmann 2002; Hofmann-Lehmann et al. 2004).

Anaplasma marginale, the causative agent for AM in cattle is transmitted by ticks or blood-feeding insects, to both, domestic and wild ruminants. Diseased animals often die of anemia and general exacerbation. In 2002, an unexpected outbreak of Anaplasmosis occurred in a trader cattle herd of the Grisons (Brülisauer et al. 2004; Dreher et al. 2004; Hofmann-Lehmann et al. 2004)

The Bluetongue virus (BTV) is particularly harmful for sheep and, depending on the breed, clinical disease is characterized by high mortality, whereas other ruminants are mostly asymptomatically infected. BTV is transmitted by biting midges of the genus *Culicoides*. Entomological studies targeted at risk areas are ongoing. An early warning system with a sentinel surveillance system in cattle is being established (Racloz et al. 2006). Investigations regarding the sero-prevalence of BT and AM in migrating red deer seasonally settled in southern regions of Switzerland and Italy might therefore provide additional insight into some risk factors for introduction of the disease to Switzerland.

Infections with pestiviruses such as bovine viral diarrhea virus (BVDV) occur in all domestic ruminants and different species of wild ruminants. BVDV is widespread among cattle in Switzerland (Rufenacht et al. 2000). Therefore, private and governmental task groups are currently planning to establish a national BVDV eradication program in cattle. In a preliminary investigation, few blood samples of Swiss red deer were tested and had antibodies against BVDV (Vogt, unpublished data). Through contamination of feedlot and environment on alpine pastures the possibility of viral transmission between the two populations is given.

The aim of this pilot study was to establish the serological status of red deer regarding BVDV, AM, and BT, and to assess the possible impact of disease dynamics with a focus on potential transmission of these diseases from red deer to cattle or vice versa.

Materials and methods

Description of study sites

The study sites were selected with regard to abundance of the targeted animal species and location of the areas along the known seasonal migration routes of red deer. A focus was given to naturally enclosed areas with cattle and red deer encountering traditional alpine pastures, especially for BVDV investigations. The selection of the sampling sites for vector-borne diseases was further influenced by aspects of potential vector distribution due to climatic conditions. The three study sites in the southern Grisons, a southeastern administrative region (canton) of Switzerland, were the Mesocco-Valley, the Poschiavo, and the Valley of Bregaglia. The forth study site, the canton of Glarus, was located in central Switzerland, and is subdivided into three mostly secluded areas by rivers. The canton of Glarus has a traditional pasture culture with numerous cattle herds spending summertime on alpine pastures.

Sample size

The required sample size was based on approximate population estimates derived from the annual hunting statistics. Exact Swiss surveys on red deer population density are scarce. According to the currant practice the annual hunting bag represents about 75 to 100% of the increment of the red deer population. Assuming disease prevalences below 1%, a sample size of 55 and 90 animals for Glarus and the study sites in Grisons, respectively, was calculated.

Sample collection procedure and material

Sample collection was conducted by hunters and gamekeepers during the two regular hunting seasons from September to December in 2004 and 2005. Hunters were advised to collect the blood of the animals shot either directly out of the heart, or from the thoracic cavity. The data sheet contained date of hunting, hunting area, sex, estimated age (by dentition), and weight of the animal.

Serology

Blood samples were centrifuged upon arrival and sera stored at −20°C until serological testing.

AM Antibodies against *A. marginale* were detected by a competitive enzyme-linked immunosorbent assay [cELISA, Veterinary Medical Research and Development (VMRD), Pullman, WA, USA] (Anonymous 2005). This test used a monoclonal antibody against the *A. marginale* surface protein 5 (MSP) and was reported to have a diagnostic sensitivity and specificity of 95.8 and 99%, respectively, in cattle (Torioni de Echaide et al. 1998). An inhibition of ≥35% as positive and a value of <25% as negative was used (Dreher et al. 2004). Positive samples were retested with an immunofluorescent antibody assay for *Anaplasma phagocytophilum* (IFA, *Ehrlichia equi* slides, VMRD, Pullman, WA, USA). EDTA-blood from correspondent positive serological samples were also tested for the presence of both, *A. marginale* and *A. phagocytophilum*, with species-

specific PCR's as described elsewhere (Pusterla et al. 1999; Hofmann-Lehmann et al. 2004).

BT Serology was performed using a commercial competitive ELISA (cELISA) from BDSL (BDSL, 25 Main Street, Dreghorn, Irvine, United Kingdom, http://www.bdsl2000.com) as a screening test according to the recommendation of the OIE (Anonymous 2004). All positive and doubtful testing samples were sent to the OIE BT reference laboratory at the Institute for Animal Health, Pirbright, UK, for confirmation. Confirmation was performed by a cELISA BDSL using soft plates instead of hard plates, and additionally by a virus neutralization test (VNT) for all BTV strains reported in Europe, namely 1, 2, 4, 9, and 16.

For the screening cELISA BDSL a test sensitivity of 100% was reported (Afshar et al. 1989). Within a sero-surveillance in cattle in Switzerland in 2003, a BDSL test specificity and its 95% confidence interval were calculated, resulting in a specificity of the cELISA performed in Swiss cattle population for individual animal samples of 96.5% (CI 96.2–96.7%).

BVD Sera from red deer were analyzed for antibodies against BVDV in a biphasic ELISA based on R1935/72 Swiss BVDV antigen. This screening test was validated for bovine serum only. Preliminary experiments demonstrated the partial cross reactivity of bovine IgG against the commercial available peroxydase labeled Rabbit-anti-deer IgG(L+H) conjugate (KPL, Kirkegaard & Perry Laboratories, Gaithersburgh, Maryland) used to detect anti BVDV antibodies from deer. Absorption (Optical density, OD) was measured 30 min after adding chromogen and netto extinction (NE) was calculated with the average OD values of the duplicates (Rufenacht et al. 2000).

Statistical analysis

All statistical calculations were performed with NCSS (Number Cruncher Statistical Systems, Kaysville, Utah, USA. http://www.NCCS.com). Differences in the apparent prevalence (AP) between regions and between sampling rounds were assessed using the χ^2 test or, when there were less than six observations per category, by the Fisher's exact test. Exact binomial 95% confidence intervals were calculated for the AP estimates. The exact location and the altitude of the animals shot were indicated on a datasheet to assess the geographical coordinates. Geographic data management was done in ArcMap 8.2 (ESRI, US).

Results

During the two sampling rounds a total of 238 blood samples were obtained from the study sites. Forty-four percent of the samples came from the canton of Glarus and

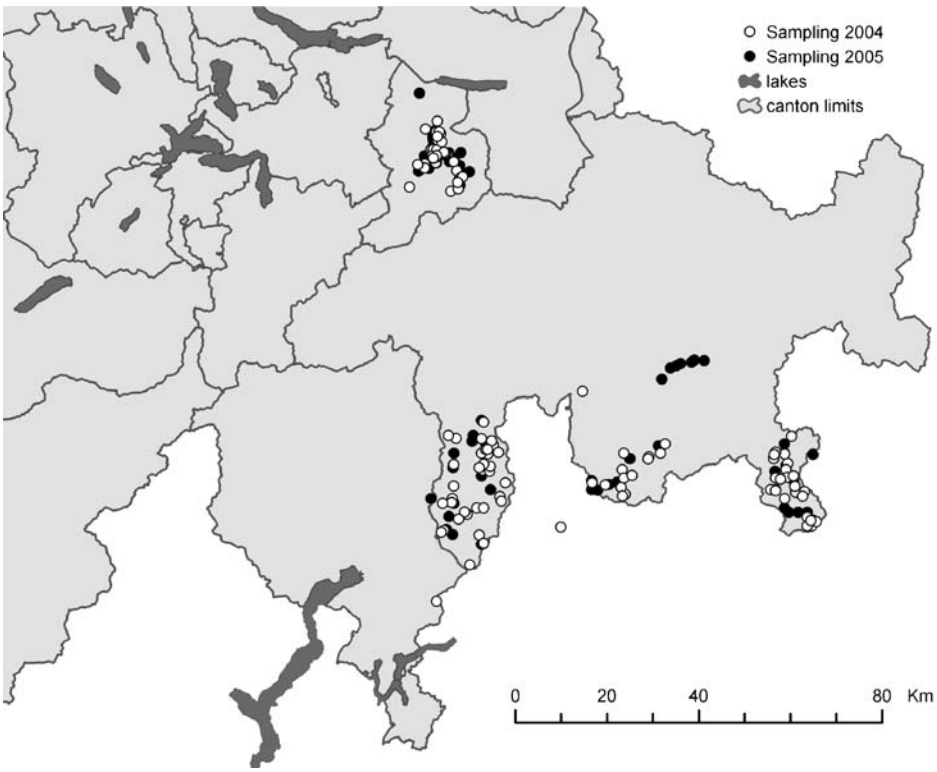

Fig. 1 Sampling areas of red deer for serological surveillance of BVD, AM and BT. Sampling periods 2004 and 2005; *white dots* sampling round 2004; *black dots* sampling round 2005

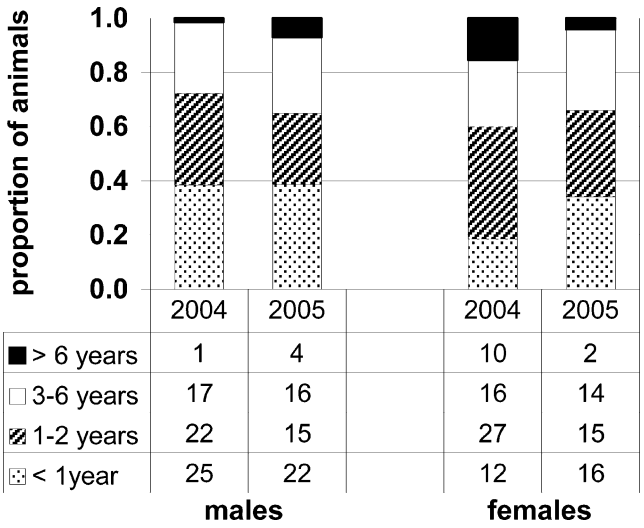

Fig. 2 Composition of age classes and sex of red deer sampled in Switzerland. 2004 $n=132$; 2005 $n=106$

56% from the three selected areas in the canton of Grisons. (Fig. 1). The distribution of sex and age classes was balanced (Fig. 2) and reflected the standard hunting bag. Animals were shot between 450 and 2,300 m over sea level.

AM Fourteen out of 238 sera showed a positive result in the cELISA. The IFAT with *A. phagocytophilum* slides carried out on ELISA-positive samples showed titers from 1:20 to 1:640. In the polymerase chain reaction (PCR) there was no evidence for the presence of *A. marginale*, whereas three of the 14 samples were positive for *A. phagocytophilum*. The AP for AM was 0% (0–1.54%) and 1.26% (0.26–3.64%) for *A. phagocytophilum*.

BT In the sampling of 2004, the BDSL test detected 15 positive samples. None of these were confirmed positive, neither in the BDSL soft plate test, nor in the VNT at the Pirbright Reference Laboratory. The AP was 0% (0–1.54%). No positive samples were reported from 2005.

BVD Out of 231 sera analyzed, 221 were negative (Nettoextinction, NE≤0.025). As an independent verification, 10 samples showing a NE>0.025 and 12 ELISA-negative sera were tested in a serum neutralization assay combined with immunostaining (Steck et al. 1980; Thur 1993). The four ELISA-positive sera, showing a NE≥ 0.08, had neutralizing titers ≥1:32. The remaining sera showed titers between 1:3 and 1:11 and were considered negative according to an arbitrary cut-off set at 1:25. The AP was thus 1.68% (0.46–4.25%).

Discussion

This investigation was designed as a pilot study with the purpose to establish the sero-prevalence of the three infectious agents of AM, BT and BVD in red deer of selected areas of Switzerland that are considered as high risk areas (Racloz et al. 2006). Serological results in wild animal are often ambiguous due to the lack of adequate diagnostic tests (Frolich et al. 2002). Thus, different statements in research projects, indicating high sero-prevalences in various deer species (white tail deer, fallow deer or mule deer), must be compared with caution, either due to species differences, or the different environmental context (Giovannini et al. 1988; Chomel et al. 1994; Van Campen et al. 2001; Krametter et al. 2004).

The results achieved for the two vector-borne diseases, AM and BT, are encouraging, although they applied only for the study areas. Up to date, no firm evidence exists for the presence or spread of the vector-borne diseases, AM and BT, in red deer or in the domestic ruminants of Switzerland (Cagienard et al. 2004; Dreher et al. 2005). Due to the fact that *A. phagocytophilum* and *A. marginale* are serologically cross reacting, only PCR will provide conclusive proof of presence or spread of *A. marginale* in the red deer population (Dreher et al. 2005).

The parallel ongoing study targeted at serological surveillance of BT in cattle is currently establishing an early warning system to detect incursions of the virus in livestock before substantial spread (Racloz et al. 2006). Additionally, extended entomological investigations by means of insect trapping are supporting the surveillance for BT.

Comparing the high BVD sero-prevalences in cattle (Rufenacht et al. 2000) to our low sero-prevalence findings in red deer, disease transmission from wild to domestic ruminants seems rather unlikely. More thorough research was carried out in Germany, revealing that stable (low) BVD sero-prevalences in red deer were independent of varying sero-prevalences in cattle in the same study area (Kleinschmidt 2004). These findings indicate that red deer is not likely to represent a relevant reservoir for the disease and for infection of cattle in Switzerland. Once the eradication program for BVD in cattle will be in finalizing stage, a resumption of the investigations might be considered and more wild ruminants should be tested in other commonly shared pastures to generate supplementary targeted results.

Under the current conditions assuming a stable red deer population size and no drastic climate change, the results achieved in our study indicated no need for a repeated large scale surveillance of red deer for the selected diseases in

Switzerland. However, in view of overlapping habitats disease awareness has to be maintained in both, domestic and wild ruminants, especially for vector-born diseases.

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