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Serological Survey for Potential Disease Agents of Free-ranging Cervids in Six Selected National Parks from Germany

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ABSTRACT: A total of 164 blood samples, collected from free-ranging red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) in six German national parks (NP) between 2000 and 2002, were assayed for antibodies against nine viral disease agents. Antibodies were only detected against the α-herpesviruses; specifically, bovine herpesvirus-1 (BHV-1) (22 of 157, 14%), cervid herpesvirus-1 (17 of 157, 10.8%), and caprine herpesvirus-1 (11 of 159, 6.9%). Titers ranged from 4 to 102. Most of the seropositive sera, and those with the highest antibody titers, were from red and roe deer in the Harz and Hochharz NP, which are connected and allow migration between the two. The distribution and specificity of antibodies detected in individual deer suggests that the three α-herpesviruses are circulating in these deer populations. No antibodies were detected against bovine viral diarrhea virus, epizootic hemorrhagic disease virus, bovine leukemia virus, bluetongue virus, foot-and-mouth disease virus, or sheep and goat poxvirus.

Key words: Bovine herpesvirus-1, caprine herpesvirus-1, cervid herpesvirus-1, free-ranging deer, Germany, national parks, serologic survey.

Germany maintains 15 national parks (NP) covering a total area of 9672 km^2, which is approximately 2.7% of the state territory. Sizes range between 30 km^2 and 4,440 km^2, offering habitat to a wide variety of mammals, including three species of cervidae, roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), and fallow deer (*Dama dama*). Because the NP are often situated relatively close to agricultural farmland, cervids within the NP are likely to be exposed to livestock pathogens (Chow and Davis, 1964; Anonymous, 2004). Some of these pathogens, including bovine herpesvirus-1 (BHV-1) and bovine virus diarrhea virus (BVDV), are already present in German livestock (e.g., Rolle and Mayr, 1978; Anonymous, 2006). Others, such as foot-and-mouth disease virus (FMDV), pose a continual threat (Forman and Gibbs, 1974; Bouma et al., 2003).

Even though economic utilization is strictly limited in NP, contact between wild and domestic animals is possible. The mutual transmission of infectious diseases between livestock and wildlife is therefore an important issue with regard to disease management. Serologic monitoring of domestic livestock and wildlife in protected areas is vital to help determine the incidence and possible patterns of spread of these infections.

Viral pathogens of livestock can be present in different deer species distributed in NP and this has been demonstrated in North America. Aguirre et al. (1995) revealed the presence of antibodies to BVDV, epizootic hemorrhagic disease virus (EHDV), parainfluenza-3 (PI-3) virus, (BHV-1), bluetongue virus (BTV), and respiratory syncytial virus (RSV) in wapiti (*Cervus elaphus canadensis*) and mule deer (*Odocoileus hemionus*) in eight national parks in the USA. Riemann et al. (1979) reported on the occurrence of antibodies to BVDV, BHV-1, PI-3 virus, and BTV in axis deer (*Axis axis*) and fallow deer in Point Reyes National Seashore, California. Positive antibodies against BTV and EHDV have also been detected in white-tailed deer (*Odocoileus virginianus*) in Mammoth Cave National Park, Kentucky (Roughton, 1975). In Canada, serosurveys have been performed in white-tailed deer from Anticosti Island, Quebec (Sadi et al., 1991), and in moose (*Alces alces*) from Cypress Hills Park, Alberta (Thorsen and Henderson, 1971); seropos-

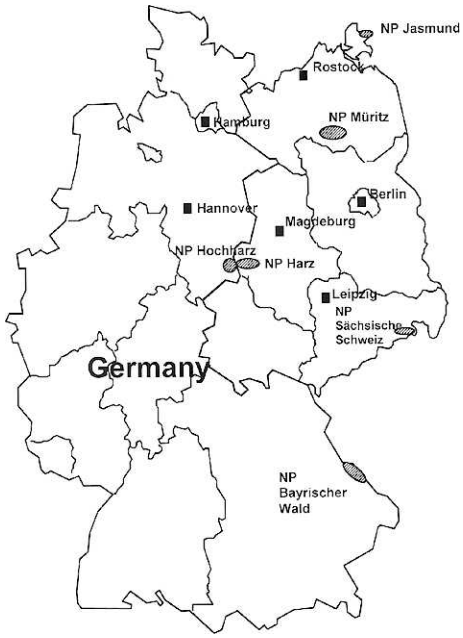

FIGURE 1. Distribution of six German national parks where blood samples were obtained.

itive reactors were found against BHV-1, BVDV and PI-3 virus.

No information currently exists related to the epidemiology of these pathogens in cervids from European national parks. The objective of this study was to determine the prevalence of antibodies to nine selected viral infections in roe deer, red deer, and fallow deer living in six of the largest NP in Germany. The selection of parks was based on size and resident deer populations.

A total of 164 blood samples were collected between 2000 and 2002 from: 1) NP Harz (51°45′N, 10°30′E); 2) NP Sächsische Schweiz (50°50′N, 14°10′E); 3) NP Müritz (53°30′N, 12°53′E); 4) NP Hochharz (51°45′N, 10°45′E); 5) NP Jasmund (54°30′N, 13°30′E), and 6) NP Bayrischer Wald (49°20′N, 13°15′E) (Fig. 1). Local deer hunters submitted blood and age and species data from harvested animals to the Institute for Zoo Biology and Wildlife Research, Berlin. The numbers and species sampled are given in Table 1. Sera were decanted and stored at −20 C. In some cases, insufficient volume or poor quality of sera did not allow testing for antibodies against all viral pathogens.

A total of 158 samples was tested for antibodies against four cytopathic BVDV strains of the antigenetic group 1 (SH9/11, Grub 313/83, NADL, and Osloss) using microneutralization tests (NT) (Frölich and Streich, 1998). Cell cultures were examined after 4 days for the presence of cytopathic effects (Frost et al., 1990) and antibody titers calculated according to Spearmann and Kärber (1985). Titers >4 were considered positive (Malmquist, 1968). Neutralization tests were performed twice for each serum and the average titer was calculated. Each test included a virus control to confirm the virus dose, a two-fold titration of a known positive control serum, a fetal calf serum negative control, and a cell control.

Sera were tested for antibodies against three different α-herpesviruses (Frölich, 1996): 157 samples were tested against BHV-1 (Cooper-type strain, USA) and the Moredun strain of cervid herpesvirus-1 (HVC-1), and samples were tested against caprine herpesvirus-1 (CapHV-1) (E/CH) using a standard NT (Ackermann et al., 1986). Antibody titers were calculated and expressed as the reciprocal of the highest dilution of serum exhibiting 50% inhibition of cytopathic effects (Horzinek, 1985). Titers ≥4 were considered positive (Ek-Kommonen et al., 1982). The NT was performed twice for each serum and the average titer was calculated.

One hundred and fifty-eight serum samples were screened for antibodies to bovine leukemia virus (BLV), using a commercial agar-gel immunodiffusion test (IDT) (Riemser Rinderleukose-Testbesteck, Riemser Arzneimittel GmbH, Riemserort, Germany) according to manufacturer's instructions. Briefly, 15 ml of a special immunodiffusion agar (0.8%, pH 7.2) was filled in 85-mm plastic dishes (NUNC GmbH, Wiesbaden, Germany). Seven depots were produced on the agar

TABLE 1. Number of deer samples ($n=164$) from different German national parks collected between 2000 and 2002.

Species	Harz	Hochharz	Sächsische Schweiz	BayrischerWald	Müritz	Jasmund	Totals
Roe deer	18	0	1	13	7	0	39
Fallow deer	0	0	0	0	11	35	46
Red deer	40	11	11	11	6	0	79
Totals	58	11	12	24	24	35	164

by a specially manufactured punch (Riemser Arzneimittel GmbH) in rosette form according to the European Community standard method. The center well was filled with antigen and two wells on opposite sides of the rosette were filled with positive control serum. Test sera were added to the remaining four wells. Gels were examined over a light box after 3 days incubation at room temperature in a moist chamber. Sera with antibodies to BLV protein gp51 formed a precipitin line of identity with that of the neighboring positive control serum.

A constant 1:5 dilution of each serum was assayed using serogroup-specific competitive enzyme linked immunosorbant assays (C-ELISA) for the presence of antibodies to BTV ($n=150$) (Anderson, 1984) and EHDV ($n=149$) (Thevasagayem et al., 1995). A positive ovine and a negative bovine control serum were included on each test plate. Sera giving percentage inhibition values equal to or greater than 50% were recorded positive.

A total of 149 serum samples was examined for the presence of antibodies against non-structural FMDV polyprotein 3ABC using a commercial kit supplied by Cedi-Diagnostics (Ceditest FMDV-NS, Cedi-diagnostics, The Netherlands) based on the assay of Sorensen et al. (1998).

Virus neutralization assays for detection of antibodies against sheep and goat poxvirus were performed in 96-well, flat-bottomed, cell culture microtiter plates. Test ($n=148$) and control sera were diluted 1:5 in DMEM, containing 10% previously screened FCS and 0.05 mg/ml gentamycin (Gentamicin®, 50 mg/ml, Gibco, Paisley, Renfrewshire, UK), and heat inactivated at 56 C for 30 min. A two-fold dilution series of each serum from 1:5 to 1:20 was subsequently prepared (100 μl/well). The positive bovine control serum used was supplied by J. A. W. Coetzer, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa. Cattle serum collected from United Kingdom was used as the negative control. An equal volume (100 μl) of a South African, field strain SA 2/94 of lumpy skin disease virus at a concentration of 100 median tissue culture infective doses ($TCID_{50}$) was added to all wells and the serum/virus mixtures incubated at 37 C for 1 hr. Primary lamb testis cells, at a concentration of 4.8×10^5/ml, were added to all wells (80 μl/well). Plates were sealed and incubated at 37 C for 14 days. The cell monolayers were examined daily for evidence of cytopathic effect. End-point titers were determined as the highest serum dilution in the serum–virus mixtures that inhibited virus growth.

Seropositive animals were identified against three α-herpesviruses, BHV-1, CapHV-1, and HVC-1, although the seroprevalence differed in the six NP (Table 2). Titers ranged from 4 to 102, the highest being recorded in adult animals located in the Harz and Hochharz NP (Table 3). The highest antibody prevalence against one or more α-herpesviruses was detected in red deer (21 of 75; 28%), whereas only six of 38 (16%) roe deer and one of 46 (2%) fallow deer tested positive. More specifically, 13 of the deer sera reacted solely against BHV-1, eight against

TABLE 2. Antibody prevalence to α-herpesviruses from free-ranging deer species in different German national parks, collected between 2000 and 2002 (**A.** distribution of seropositive reactors in each national park; **B.** overall distribution of seropositive reactors in each species).

A

	Harz		Hochharz	Sächsische Schweiz		Bayrischer Wald		Müritz			Jasmund
Antigens[a]	Roe deer	Red deer	Red deer	Roe deer	Red deer	Roe deer	Roe deer	Red deer	Fallow deer	Red deer	Fallow deer
BHV-1	4/17 (24%)[b]	11/39 (28%)	5/10 (50%)	0/1	0/10	0/13	0/8	0/7	0/11	1/6 (17%)	1/35 (3%)
CapHV-1	0/17	5/40 (13%)	3/11 (27%)	0/1	1/10 (10%)	0/13	1/8 (12.5%)	1/7 (14%)	0/11	0/6	0/35
HVC-1	1/17 (6%)	7/39 (18%)	3/10 (30%)	0/1	1/10 (10%)	1/13 (8%)	3/8 (37.5%)	0/7	0/11	1/6 (17%)	0/35

B

Antigens	Red deer	Roe deer	Fallow deer	Totals
BHV-1	17/73 (23.3%)	4/38 (10.55)	1/46 (2.2%)	22/157 (13.9%)
CapHV-1	10/75 (13.3%)	1/38 (2.6%)	0/46	11/159 (6.9%)
HVC-1	15/73 (20.5%)	2/38 (5.2%)	0/46	17/157 (10.8%)

[a] BHV-1 = bovine herpesvirus-1, CapHV-1 = caprine herpesvirus-1, HVC-1 = cervid herpesvirus-1.

[b] Number positive/Number tested (% positive).

TABLE 3. Inverse neutralization antibody titers of α-herpesviruses in 35 deer from different German national parks (NP).

Deer species	NP	CapHV-1[a]	HVC-1[b]	BHV-1[c]	Age
Roe deer	Harz	—	11	—	yearling
Roe deer	Harz	—	—	16	fawn
Roe deer	Harz	—	—	17	fawn
Roe deer	Harz	—	—	6	yearling
Roe deer	Harz	—	—	10	yearling
Red deer	Harz	—	14	—	adult
Red deer	Harz	32	42	102	adult
Red deer	Harz	—	—	7	fawn
Red deer	Harz	13	14	—	yearling
Red deer	Harz	—	—	27	fawn
Red deer	Harz	58	64	22	adult
Red deer	Harz	—	—	14	fawn
Red deer	Harz	—	8	—	fawn
Red deer	Harz	—	—	6	adult
Red deer	Harz	7	—	30	yearling
Red deer	Harz	16	8	8	adult
Red deer	Harz	—	16	7	adult
Red deer	Harz	—	—	7	fawn
Red deer	Harz	—	—	16	adult
Red deer	Sächsische Schweiz	29	—	—	fawn
Red deer	Sächsische Schweiz	—	5	—	adult
Roe deer	Müritz	28	—	—	fawn
Red deer	Müritz	—	—	35	adult
Red deer	Müritz	—	5	—	adult
Red deer	Hochharz	—	14	28	fawn
Red deer	Hochharz	—	—	16	adult
Red deer	Hochharz	61	102	61	adult
Red deer	Hochharz	—	—	14	yearling
Red deer	Hochharz	45	90	30	adult
Red deer	Hochharz	5	[d]nd	nd	fawn
Fallow deer	Jasmund	—	—	13	fawn
Roe deer	Bayrischer Wald	—	4	—	yearling
Red deer	Bayrischer Wald	—	7	—	adult
Red deer	Bayrischer Wald	5	14	—	adult
Red deer	Bayrischer Wald	—	7	—	adult
Totals		11	17	22	

[a] CapHV-1 = caprine herpesvirus 1.

[b] BHV-1 = bovine herpes-virus 1.

[c] HVC-1 cervid herpesvirus 1.

[d] nd = not determined, insufficient volume available.

HVC-1, and two against CapHV-1. Interestingly, only five of the sera gave positive antibody reactions against all three α-herpesviruses; with these animals, the highest titer was observed against BHV-1 in one, HCV-1 in three, and CapHV-1 (although weak) in the remaining deer. Five sera reacted against two of the α-herpesviruses; two against BHV-1 and HVC-1, two against HVC-1 and CapHV-1, and one against BHV-1 and CapHV-1. No antibodies were detected against BVDV, EHDV, BLV, BTV, FMDV, or sheep and goat poxvirus.

Although restricted to Germany, this is the first survey of European NP for the presence of antibodies to viral diseases in cervids. Results from previous studies have revealed antibodies to some of these agents in free-ranging deer elsewhere in

Germany and in other European countries (e.g., Weber et al., 1978; Thiry et al., 1988; Liebermann et al., 1989; Thiry et al., 1992; Frölich, 1996; Pospisil et al., 1996; Lillehaug et al., 2003).

Results presented in this report for the three α-herpesviruses are in accordance with those reported previously for free-ranging deer elsewhere in Germany (Dedek and Loepelmann, 1988; Frölich, 1996; Müller et al., 1996, 1997). Serological cross-reactions between the α-herpesviruses are well-documented (Nixon et al., 1988; Martin et al., 1990) but it has been demonstrated that the highest antibody titers occur against the specific infecting agent.

Interestingly, monospecific antibody responses to BHV-1 were detected in sera from several of the red and roe deer that lived in the Harz and Hochharz NP (Table 3). These parks are connected (Fig. 1) and red deer migrate between the two NP, which might also account for the similar distribution and levels of antibodies recorded against the α-herpesviruses. Transmission of BHV-1 from cattle that are in close proximity to these parks is one possible explanation. This supposition is supported by the work of Lawman et al. (1978) who detected BHV-1 positive reactions in red deer, particularly in areas where BHV-1 was prevalent in cattle. These findings show the apparent susceptibility of deer to BHV-1 and highlight the possible risk of "spill over" between domestic and wild species. The absence of antibodies to BHV-1 in deer in the Bayrischer Wald NP might be due to the absence of BHV-1 positive cattle in the surrounding area or lack of close contact.

The fact that most seropositive reactors to all three α-herpesviruses were found in red deer corresponds to the results of Kokles et al. (1988) and Pospisil et al. (1996) who detected α-herpesviruses in 68% of red deer in the Czech Republic. Most of the fawns and yearlings tested in this study reacted against only one of the α-herpesviruses and in particular BHV-1. Most of the seropositive adult deer on the other hand, recorded antibodies against two or all three α-herpesviruses. This is not unexpected, because the adults would have had more opportunity for exposure than younger animals. Because red deer live in large groups throughout the year and migrate between different regions (Nowak, 1999), their exposure to pathogens might be higher as compared to roe deer, which have small home ranges and are seasonally territorial or live in small groups in winter (Hewison et al., 1998). Alternatively, red deer might be more susceptible to α-herpesviruses than roe deer.

The apparent low prevalence and distribution of antibodies (Table 2) against the α-herpesviruses in the remaining parks (Sächsische Schweiz, Müritz, Jasmund, and Bayrischer Wald) is probably a reflection of their location, their possible isolation from domestic animals and/or the relatively small numbers of deer sampled. It is noteworthy that in a previous study on α-herpesviruses in free-living German deer (Frölich, 1996), most seropositive deer and the highest titers were against CapHV-1. This could also add support to our conclusion that different α-herpesviruses might be circulating within the different deer populations in Germany.

No antibodies were detected to any of the other agents assayed in this study. The negative results for EBLV and FMDV confirm the results of previous investigations of cervids in Germany (Dedek et al., 1987; Dedek and Loepelmann, 1988; Müller et al., 1996; Müller et al., 1997; Stubbe et al., 1996; Mouchantat et al., 2005) for the absence of infection in cervids throughout Germany. Although no antibodies were detected against pestiviruses, seropositive reactors have been detected previously in deer (Dedek et al., 1988; Dedek and Loepelmann, 1988; Frölich, 1995; Müller et al., 1996, 1997; Stubbe et al., 1996) and BVDV is present in livestock situated close to NP Hochharz, NP Müritz, and NP Sächsische Schweiz.

In conclusion, the results of this study show that free-ranging cervids from the six NP that were sampled have been exposed to three antigenically different α-herpesviruses, with the highest seroprevalence being recorded against BHV-1. Most seropositive reactors and the highest titers were found among red deer. Cross-reactions between the α-herpesviruses have been reported; however, the distribution of antibody recorded here in the widely separated NP does not suggest a consistent pattern that could be solely attributed to this phenomenon. The higher seroprevalences recorded in at least two of the NP suggests a possible "spill over" of virus from BHV-1 positive cattle. With respect to wildlife disease management in NP, our results highlight the possible risks of transmission of infectious and contagious pathogens from wildlife to domestic livestock and visa versa. To help protect these valuable resources, efforts should be made to keep the cervids in NP isolated from domestic livestock and to conduct regular serological surveillance of both groups.

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