

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

Authors: Frölich, Kai, Hamblin, Chris, Parida, Satya, Tuppurainen, Eeva, and Schettler, Elvira

Source: Journal of Wildlife Diseases, 42(4) : 836-843

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-42.4.836>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

Kai Frölich,^{1,3} Chris Hamblin,² Satya Parida,² Eeva Tuppurainen,² and Elvira Schettler¹ ¹ Institute for Zoo and Wildlife Research Berlin, Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany; ² Institute for Animal Health, Ash Road, Pirbright, Surrey GU24 0NF, United Kingdom; ³ Corresponding author (email: froelich@izw-berlin.de)

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 km², which is approximately 2.7% of the state territory. Sizes range between 30 km² and 4,440 km², 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 Spearman 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 | Bayrischer Wald | 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 μ l/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 μ l) of a South African, field strain SA 2/94 of lumpy skin disease virus at a concentration of 100 median tissue culture infective doses (TCID₅₀) 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 μ l/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 | | | | | | | | | | | |
|-----------------------|-------------------------|-------------|------------|----------|--------------------|-----------|-----------------|-----------|-------------|-----------|-------------|
| Antigens ^a | Harz | | Hochharz | | Sächsische Schweiz | | Bayrischer Wald | | Müritztz | | Jasmund |
| | Roe deer | Red deer | Roe deer | Red deer | Roe deer | Red 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 | Roe deer | | Fallow deer | |
| | Totals | | Totals | |
| BHV-1 | 17/73 (23.3%) | 4/38 (10.5%) | 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 Bayerischer 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, Müritzt, Jasmund, and Bayerischer 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 Müritzt, 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.

LITERATURE CITED

- ACKERMANN, M., A. E. MELTZER, H. McDONAGH, L. BRUCKNER, H. K. MÜLLER, AND U. KIHM. 1986. Stellen nichtbovine Paarhufer ein IBR-Virus-Reservoir dar? *Schweizer Archiv für Tierheilkunde* 128: 557–573.
- AGUIRRE, A. A., D. E. HANSEN, E. E. STARKEY, AND R. G. MCLEAN. 1995. Serologic survey of wild cervids for potential disease agents in selected national parks in the United States. *Preventive Veterinary Medicine* 21: 313–322.
- ANDERSON, J. 1984. Use of monoclonal antibody in a blocking ELISA to detect antibodies to bluetongue virus. *Journal of Immunological Methods* 74: 139–149.
- ANONYMOUS. 2004. Nationalparke in Deutschland. *Europac Deutschland*, Berlin, Germany, 36 pp.
- . 2006. *Annual animal disease status, Germany 2000–2004. OIE Handistatus II. Paris, OIE*. <http://www.oie.int/hs2/report.asp>. Accessed September 2006.
- BOUMA, A., A. R. W. ELBERS, A. DEKKER, A. DE KOEIJER, C. BARTELS, P. VELLEMA, P. VAN DER WAL, E. M. A. VAN ROOIJ, F. H. PLUIMERS, AND M. C. M. DE JONG. 2003. The foot-and-mouth disease epidemic in the Netherlands in 2001. *Preventative Veterinary Medicine* 57: 155–166.
- CHOW, T. L., AND R. W. DAVIS. 1964. The susceptibility of mule deer to infectious bovine rhinotracheitis. *American Journal of Veterinary Research* 25: 518–519.
- DEDEK, J., AND H. LOEPELMANN. 1988. Ergebnisse flächendeckender serologischer Untersuchungen beim Rot-, Reh-, Dam- und Muffelwild in einem Bezirk der DDR. *Internationales Symposium über die Erkrankungen der Zoo- und Wildtiere* 30: 63–69.
- , ———, M. MÜLLER, AND S. DADEMARSCH. 1987. Serologische Untersuchungen bei einheimischen Wildwiederkäuern (Rot-, Reh-, Dam-, Muffelwild) auf enzootische Rinderleukose. *Monatshefte für Veterinärmedizin* 42: 784–785.
- , ———, R. KOKLES, C. KRETZSCHMAR, M. MÜLLER, AND H. BERGMANN. 1988. Ergebnisse serologischer Untersuchungen auf Antikörper gegen das Virus der bovinen Virusdiarrhoe/Mucosal disease beim Rot-, Reh-, Dam- und Muffelwild. *Monatshefte für Veterinärmedizin* 43: 63–65.
- EK-KOMMONEN, C., P. VEIJALAINEN, M. RANTALA, AND E. NEUVONEN. 1982. Neutralizing antibodies to bovine herpesvirus 1 in reindeer. *Acta Veterinaria Scandinavica* 23: 565–569.
- FORMAN, A. J., AND E. P. J. GIBBS. 1974. Studies in foot-and-mouth disease in British deer (red, fallow and roe). I. Clinical disease. *Journal of Comparative Pathology* 84: 215–220.
- FRÖLICH, K. 1995. Bovine virus diarrhea and mucosal disease in free-ranging and captive deer (Cervidae) in Germany. *Journal of Wildlife Diseases* 31: 247–250.
- . 1996. Seroepizootiologic investigations of herpesviruses in free-ranging and captive deer (Cervidae) in Germany. *Journal of Zoo and Wildlife Medicine* 27: 241–247.
- , AND W. Y. STREICH. 1998. Zoological evidence of bovine virus diarrhea in free ranging rabbits in Germany. *Journal of Wildlife Diseases* 32: 173–178.
- FROST, J. W., I. WESTESTPHÄLING, AND H. KRAUSS. 1990. Seroepidemiologische Untersuchung bei Schafen in Süd- und Mittelhessen zur Verbreitung von Antikörpern gegen Border-Disease/BVD-Virus. *Tierärztliche Umschau* 46: 533–536.
- HEWISON, A. J. M., J. P. VINCENT, AND D. REBY. 1998. Social organisation of European roe deer. *In The European roe deer: The biology of success*, R. Andersen, P. Duncan, and J. D. C. Linnell (eds.). Scandinavian University Press, Oslo, Norway, pp. 189–221.
- HORZINEK, M. C. 1985. *Kompandium der allgemeinen Virologie*, 2. Auflage, Verlag Paul Parey, Berlin, Germany, 159 pp.
- KOKLES, R., J. DEDEK, AND H. LOEPELMANN. 1988. Serologische Untersuchungen auf Infektionen mit dem Virus der Infektiösen bovinen Rhinotracheitis/Infektiösen pustulösen Vulvovaginitis

- und dem Parainfluenza-3-Virus bei Rot-, Reh-, Dam- und Muffelwild. Monatshefte für Veterinärmedizin 43: 60–63.
- LAWMAN, J. P., D. J. EVANS, E. P. J. GIBBS, A. MCDIARMID, AND L. EOWE. 1978. A preliminary survey of British deer for antibody to some virus disease of farm animals. British Veterinary Journal 134: 85–91.
- LIEBERMANN, H., T. TABBAA, J. DEDEK, H. LOEPELMANN, I. STUBBE, AND H.-J. SELBITZ. 1989. Serologische Untersuchungen auf ausgewählte Virusinfektionen bei Wildwiederkäuern in der DDR. Monatshefte für Veterinärmedizin 44: 380–382.
- LILLEHAUG, A., T. VIKOREN, I.-L. LARSEN, J. AKERSTEDT, J. THARALDSEN, AND K. HANDELAND. 2003. Antibodies to ruminant alpha-herpesviruses and pestiviruses in Norwegian cervids. Journal of Wildlife Diseases 39: 779–786.
- MALMQUIST, W. A. 1968. Bovine viral diarrhea-mucosal disease. Etiology, pathogenesis and applied immunity. Journal of the American Veterinary Medical Association 156: 763–770.
- MARTIN, W. B., G. CASTRUCCI, F. FRIGERI, AND M. FERRARI. 1990. A serological comparison of some animal herpesviruses. Comparative Immunology Microbiology, and Infectious Diseases 13: 75–84.
- MOUCHANTAT, S., B. HAAS, W. KUTZ, K. POHLMAYER, AND K. FRÖLICH. 2005. Absence of antibodies to foot-and-mouth disease in free-ranging roe deer from selected areas of Germany 2001–2002. Journal of Wildlife Diseases 41: 599–605.
- MÜLLER, T., M. KRAMER, J. TEUFFERT, D. BEIER, K. ZIEDLER, AND C. POSSARDT. 1996. Vorkommen ausgewählter viraler Erkrankungen beim einheimischen Schalenwild im Land Brandenburg. Beiträge zur Jagd- und Wildforschung 21: 183–190.
- , AND D. BEIER. 1997. A serological screening on the occurrence of antibodies against selected bovine and ovine viral diseases in roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*) in Brandenburg. Zeitschrift für Jagdwissenschaften 43: 166–175.
- NIXON, P., S. EDWARDS, AND H. WHITE. 1988. Serological comparisons of antigenically related herpesviruses in cattle, red deer and goats. Veterinary Research Communications 12: 355–362.
- NOWAK, R. M. 1999. Walker's Mammals of the World, 6th Edition, Vol. II. John Hopkins University Press, Baltimore, Maryland, 1936 pp.
- POSPISIL, Z., R. VYVLECKA, P. CIHAL, P. LANY, AND D. ZENDULKOVA. 1996. Demonstration of antibodies to herpes virus in the sera of red deer (*Cervus elaphus*) imported into the Czech Republic. Veterinarni Medicina 41: 279–282.
- RIEMANN, H. P., R. RUPPANNER, P. WILLEBERG, C. E. FRANTI, W. H. ELLIOT, R. A. FISHER, O. A. BRUNETTI, J. H. AHO, JR., J. A. HOWARTH, AND D. E. BEHYMER. 1979. Serologic profile of exotic deer at Point Reyes National Seashore. Journal of the American Veterinary Medical Association 175: 911–913.
- ROLLE, M., AND A. MAYR. 1978. Mikrobiologie, Infektions und Seuchenlehre. Enke, Stuttgart, Germany, 835 pp.
- ROUGHTON, R. D. 1975. An outbreak of a hemorrhagic disease in white-tailed deer in Kentucky. Journal of Wildlife Diseases 11: 177–186.
- SADI, L., R. JOYAL, M. ST. GEORGES, AND L. LAMONTAGUE. 1991. Serologic survey of white-tailed deer on Anticosti Island, Quebec, for bovine herpesvirus 1, bovine viral diarrhea, and parainfluenza 3. Journal of Wildlife Diseases 27: 569–577.
- SORENSEN, K. J., K. G. MADSEN, E. S. MADSEN, J. S. SALT, J. NQINDI, AND D. K. J. MACKAY. 1998. Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. Archives of Virology 143: 1461–1476.
- SPEARMANN, R., AND G. KÄRBER. 1985. Das virus als partikel. In Kompendium der allgemeinen virologie, 2nd Edition, M. C. Horzinek (ed.). Paul Parey, Berlin, Germany, pp. 22–23.
- STUBBE, I., W. STUBBE, H. PIEGERT, AND B. GEHRMANN. 1996. Weitere seroepidemiologische Untersuchungen an heimischen Wildtieren. Beiträge zur Jagd- und Wildforschung 21: 191–197.
- THEVASAGAYAM, J. A., P. P. MERTENS, J. N. BURROUGHS, AND J. ANDERSON. 1995. Competitive ELISA for the detection of antibodies against epizootic haemorrhagic disease of deer virus. Journal of Virological Methods 55: 417–425.
- THIRY, E., M. VERCOUTER, J. DUBUISSON, J. BARRAT, C. SEPULCHRE, C. GERARDY, C. MEERSCHAERT, B. COLLIN, J. BLANCOU, AND P. P. PASTORET. 1988. Serological survey of herpesvirus infections in wild ruminants of France and Belgium. Journal of Wildlife Diseases 24: 268–273.
- , M. BORRENS, J. BARRAT, J. BLANCOU, AND P.-P. PASTORET. 1992. Seroepidemiological survey of herpesvirus infections in wild ruminants in France. Gibier Faune Sauvage 9: 87–91.
- THORSEN, J., AND J. P. HENDERSON. 1971. Survey for antibody to infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), and parainfluenza 3 (PI3) in moose sera. Journal of Wildlife Diseases 7: 93–95.
- WEBER, A., J. PAULSEN, AND H. KRAUS. 1978. Seroepidemiologische Untersuchungen zum Vorkommen von Infektionskrankheiten bei einheimischem Schalenwild. Praktischer Tierarzt 59: 353–358.

Received for publication 6 September 2005.