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SEROSURVEY FOR ANTIBODIES TO MALIGNANT CATARRHAL FEVER-ASSOCIATED VIRUSES IN FREE-LIVING AND CAPTIVE CERVIDS IN GERMANY

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ABSTRACT: A total of 486 serum samples collected from several species of both free-living and captive cervids in Germany was examined for antibodies against malignant catarrhal fever (MCF)-associated viruses (MCFV) by a competitive-inhibition enzyme-linked immunosorbent assay (CI-ELISA). Eleven (2%) of these samples were positive for antibodies against MCFV. Among 157 serum samples collected from 16 different species of captive deer including four (7%) of 54 fallow deer and one (7%) of 14 sika deer (*Cervus nippon*) were seropositive. Among 329 samples from three species of free-ranging deer, including 253 roe deer (*Capreolus capreolus*), 22 red deer (*Cervus elaphus*) and 54 fallow deer (*Cervus dama*), only fallow deer were antibody-positive. Of the 25 fallow deer samples collected between 1990 and 1993, four (16%) were seropositive. Among 29 free-ranging fallow deer samples collected in the hunting period 1996–1997, antibodies to MCFV were detected in two (7%) of these sera. All of these fallow deer samples were collected from a circumscribed area in northern Germany. In the same area a high seroprevalence (72%) to MCFV was observed in domestic sheep ($n = 50$). Among 20 sheep samples (buffy coat) and 15 fallow deer samples (spleen or lymph nodes) examined for ovine herpesvirus 2 (OvHV-2) by PCR, all 20 sheep samples examined were OvHV-2 positive, but all of the 15 fallow deer samples, including seven seropositive deer, were OvHV-2 negative.

Key words: Captive deer, *Cervus dama*, epizootiology, free-ranging deer, malignant catarrhal fever, ovine herpesvirus 2, polymerase chain reaction, serosurvey.

INTRODUCTION

Malignant catarrhal fever (MCF) affects many species of ruminants (Seal et al., 1989; Murphy et al., 1994) and is considered to be a major threat to the development of deer farming (Reid, 1992). Based on the reservoir ruminant species from which the causative viruses arise, the two major epizootiological entities of the disease that have been described are wildebeest-associated (WA) and sheep-associated (SA) MCF. The etiologic agent for WA-MCF has been isolated, characterized as a gammaherpesvirus, and named alcelaphine herpesvirus 1 (AHV-1) (Plowright et al., 1960; Bridgen et al., 1989) while the putative SA-MCF agent has not yet been isolated (Reid, 1992). Based on its antigenic and base-sequence homology to AHV-1, the putative agent of the SA-MCF has been tentatively classified as ovine herpesvirus 2 (OvHV-2) (Roizman, 1992). Ma-

lignant catarrhal fever has been described in several species of deer (Westbury, 1984), and cervids are generally regarded as highly susceptible to MCF (Plowright, 1986; Buxton, 1988). However, the cause of MCF in deer is not definitely confirmed in most cases, although it is thought to be a virus carried by clinically normal sheep (Buxton, 1988).

The range of clinical signs observed in MCF affected deer has been diverse (Westbury, 1984; Blake et al., 1990). The disease tends to be peracute or acute, with animals succumbing before the more florid lesions, characteristic of protracted cases, develop (Reid and Buxton, 1989). However, MCF in deer also can be present as subacute or chronic disease, the clinical signs becoming progressively more marked with duration of illness (Buxton, 1988).

In farmed deer, MCF is usually sporadic

but occasionally occurs epizootically, causing significant losses (Pierson et al., 1974; Westbury, 1984). Such epidemics of MCF in farmed deer have been reported for several species (e.g., red deer, *Cervus elaphus*; sika deer, *Cervus nippon*; Pere David's deer, *Elaphurus davidianus*; rusa deer, *Cervus timorensis*) from the UK, Australia and New Zealand where it is recognized as the most serious infectious disease (Buxton, 1988). In continental Europe, MCF in captive deer was, for example, observed in two outbreaks in Denmark (Krogh and Jensen, 1988). One of the two outbreaks started in a quarantine of 27 red deer imported from Germany. Sixteen died and MCF was diagnosed in all the six animals that were submitted for post-mortem examination. In the second outbreak nine of 11 sika deer died on a farm in Denmark and in eight of these animals MCF was diagnosed (Krogh and Jensen, 1988).

In continental Europe, only a single outbreak of MCF in free-living deer has been reported. The disease was diagnosed by pathological findings in two free-ranging moose (*Alces alces*) from Sweden, which showed abnormal behavior and nervous signs. The two moose in the study had previous contact with sheep (Warsame and Steen, 1989).

Limited data are available on the transmissibility of SA-MCF agent in deer, and there is only circumstantial evidence indicating species-specific differences in susceptibility. Pere David's deer, white-tailed deer (*Odocoileus virginianus*), sika deer and sambar deer (*Cervus unicolor*) seem to be the most susceptible to clinical MCF, while red deer and elk (*Cervus elaphus canadensis*) are moderately susceptible and fallow deer appear resistant (Mackintosh, 1993). This corresponds with findings by Hunter (1981) in which sika deer seem to be very susceptible to MCF and high mortality rates have been reported in this species. Also, according to English (1981) fallow deer seem to be relatively resistant to MCF as it has never been positively di-

agnosed in fallow deer even when these animals have been in contact with other species of deer in which MCF has occurred. However, in New Zealand MCF has been diagnosed in one fallow deer by pathological examination (Horner, 1980).

Our objective in this study was to determine whether or not free-ranging cervids in Germany, especially fallow deer, can be naturally infected by MCF-associated viruses (MCFV) (i.e. AHV-1 and OvHV-2) and to determine the seroprevalence to MCFV in both free-ranging and captive deer.

MATERIALS AND METHODS

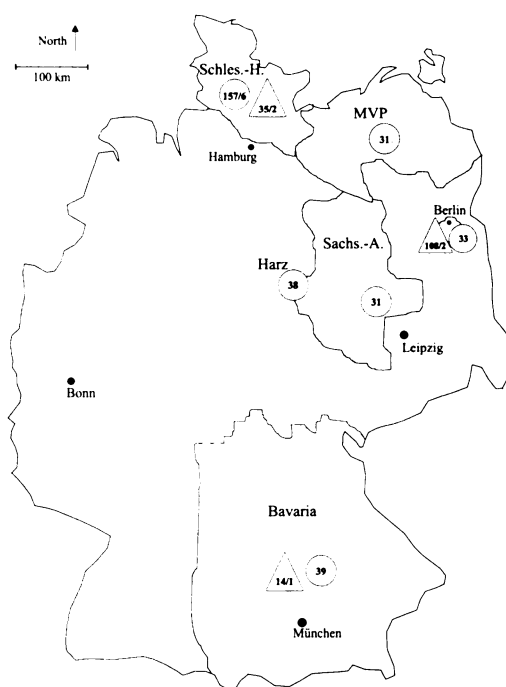
Hunters provided blood samples, spleens and lymph nodes, together with information on prepared forms, indicating the species, age and sex of deer. Cervids up to 2-yr-old (yearlings) and fawns were considered as juveniles; adult animals were >2-yr-old. The samples originated from hunting areas of Germany in Schleswig-Holstein (54°30' to 54°54'N, 9°00' to 9°38'E), Bavaria (48°00' to 48°50'N, 9°30'E to 13°10'E), Mecklenburg-Vorpommern (53°20' to 53°30'N, 12°40' to 13°00'E), Harz (51°40' to 52°00'N, 10°20' to 11°10'E), Sachsen-Anhalt (51°30' to 51°50'N, 12°15' to 12°20'E) and from forests in Berlin (52°30'N, 13°20'E). Additionally, we evaluated serum samples from nine wildlife parks from Schleswig-Holstein and Bavaria and the two zoos in Berlin (Berlin Zoo and Tierpark Berlin Friedrichsfelde) (Table 1 and Fig. 1).

In the first step we collected 457 usable blood samples from free-ranging ($n = 300$) and captive ($n = 157$) cervids between 1979 and 1996. In the second step we addressed the origin of seropositive reactions in fallow deer and, therefore, we collected 29 blood samples from free-ranging fallow deer exclusively from northern Germany in the 1996-97 hunting season. These samples originated from a circumscribed hunting area in Schleswig-Holstein (54°34' to 54°40'N, 9°28' to 9°38'E). This area was characterized by a relatively high density of sheep (0.3 sheep/ha cultivated area) and simultaneously a high population of fallow deer (8 individuals/100 ha). In addition, we selected usable spleen and lymph node samples of 15 free-ranging and captive fallow deer from Schleswig-Holstein and collected blood samples of 50 adult domestic sheep (all >1-yr-old) from three different flocks from the same area in April 1997.

All sera were tested for specific antibodies to

TABLE 1. Distribution of deer samples in different areas of Germany, 1979–1997, expressed as the number of samples tested (number of samples seropositive for MCFV).

Deer	Free-ranging deer						Captive deer			Total
	Schles.H. ^a	Bavaria	Berlin	Harz	Sachs.-A. ^b	MVP ^c	Wildlife parks	Berlin zoo	Tpk.B. ^d	
Roe deer	100	20	33	38	31	31	1	—	—	254
Fallow deer	54 (6)	—	—	—	—	—	35 (3)	1	18 (1)	108 (10)
Red deer	3	19	—	—	—	—	14	3	30	69
Sika deer	— ^e	—	—	—	—	—	—	1	13 (1)	14 (1)
Other cervid species ^f	—	—	—	—	—	—	—	13	28	41
Total	157 (6)	39	33	38	31	31	50 (3)	18	89 (2)	486 (11)

^a Schles.-H. = Schleswig-Holstein.^b Sachs.-A. = Sachsen-Anhalt.^c MVP = Mecklenburg-Vorpommern.^d Tpk.B. = Tierpark Berlin Friedrichsfelde.^e — = no samples taken.^f Buchar deer (*Cervus elaphus bactrianus*) (n = 6), Thorold's deer (*Cervus albirostris*) (n = 2), eld deer (*Cervus eldi*) (n = 4), axis deer (*Axis axis*) (n = 4), barasingha deer (*Cervus duvauceli*) (n = 8), marsh deer (*Odocoileus dichotomus*) (n = 2), pampas deer (*Odocoileus bezoarticus*) (n = 1), Pere David's deer (n = 3), white-tailed deer (n = 3), American moose (*Alces alces*) (n = 5), caribou (*Rangifer tarandus*) (n = 2), and tufted deer (*Elaphodus cephalophus*) (n = 1).FIGURE 1. Distribution of deer samples in different areas of Germany indicating the number of samples tested/number of samples seropositive for malignant catarrhal fever-associated viruses where Δ represents captive deer, and $\circ$ represents free-ranging deer. Abbreviations on the map are as follows: Schles.-H. = Schleswig-Holstein, Sachs.-A. = Sachsen-Anhalt, and MVP = Mecklenburg-Vorpommern.

MCFV by competitive-inhibition enzyme-linked immunosorbent assay (CI-ELISA) (Li et al., 1994). Spleens or lymph nodes from 15 fallow deer (7 seropositive and 8 seronegative animals) and 20 representative buffy coat samples from the 50 sheep samples were examined for OvHV-2 DNA using a semi-nested polymerase chain reaction (PCR) (Baxter et al., 1993). The cycling conditions were modified as 5 min. at 99 C prior to addition of enzyme at 60 C and 39 cycles for the first and the nested run. Tissue samples from clinically affected Pere David's deer and cattle served as positive controls.

RESULTS

Of 486 blood samples collected from different free-ranging and captive species of cervids, 11 (2%) samples were positive for MCFV antibodies. Ten of these were collected from fallow deer and one serum originated from a sika deer (Table 1 and Fig. 1). Six of these animals were yearlings, two were >2-yr-old, and the age of the remaining three individuals was unknown.

Of 157 sera collected from 16 different species of captive cervids, four (7%) of 54 fallow deer and one (7%) of 14 sika deer had antibodies against MCFV. Three of the positive fallow deer sera were collected from two different wildlife parks and one from the Tierpark Berlin Friedrichs-

felde. None of the cervid sera collected from the Berlin Zoo was seropositive.

Of 329 sera collected from three species of free-ranging deer, including roe deer, red deer and fallow deer from different regions of Germany, only fallow deer were found positive for antibodies to MCFV. Of 25 samples from fallow deer collected between 1990 and 1993, four (16%) were seropositive. Among 29 samples from fallow deer collected in the hunting period 1996–1997, antibodies against MCFV were detected in two (7%) sera. All of these fallow deer originated from the above-mentioned circumscribed area in northern Germany (Fig. 1). Among 50 domestic sheep sera collected from the same area 36 (72%) were positive for MCFV antibodies.

In the PCR, 20 of 20 examined sheep samples were positive for OvHV-2, but none of the 15 fallow deer samples, including the seven seropositive deer. Since contact with wildebeest could be excluded in the area, a PCR for AHV-1 was not included in this study. Sixteen of these 20 PCR-positive sheep were also positive in CI-ELISA.

DISCUSSION

The serological survey reveals the evidence that MCFV infection occurs in both free-ranging and captive cervids in Germany. In free-ranging cervids, antibodies against MCFV were found exclusively in fallow deer and only in a circumscribed hunting area in northern Germany, where clinical cases of MCF in cattle have been reported over the past years (P. Steinhagen, pers. comm.). In captive deer, MCFV specific antibodies were present in fallow deer as well as in a sika deer.

This is the first detection of antibodies, i.e., evidence for natural exposure to MCFV in free-ranging and captive fallow deer. According to English (1981) and Mackintosh (1993) fallow deer appear to be relatively resistant to clinical MCF although they have been extensively farmed and kept in contact with species that have developed the disease or with MCFV-car-

rier species such as domestic sheep. Also in the present study the hunters did not observe clinical signs in any exposed fallow deer. Antibodies against MCFV in apparently normal white-tailed deer and mule deer have previously been described by Li et al. (1996).

Interestingly, even though a large number of roe deer samples was examined from different areas in Germany, antibodies could not be detected in any of them. However, it has been reported that roe deer are clinically-susceptible to MCF both naturally and experimentally (Hänichen and Mannl, 1984; Reid et al., 1986). The reason for our observation is not clear. It is possible that these animals had not been exposed to MCFV or that the infection causes a high mortality leaving few survivors with antibodies. Moreover, it cannot be excluded that certain species of deer failed to respond in the CI-ELISA.

The reason for the failure to detect OvHV-2 specific DNA by PCR, especially in the seven antibody-positive fallow deer, also remains unclear. The negative results may reflect that the virus had been cleared from the animals or was not present at detectable levels in the examined tissues of healthy latently infected fallow deer. The existence of a yet unidentified herpesvirus in fallow deer, cannot be completely excluded. However, OvHV-2 is being regarded as the most likely virus, which could have caused the serological response detected in these fallow deer.

Deer are susceptible to wildebeest- and sheep-associated MCF (Westbury, 1984). The cause of cervid MCF, apart from cases of WA-MCF, has not been determined in most cases, although it is infectious, since the disease can be transmitted experimentally from deer to deer, to cattle and to rabbits (Buxton and Reid, 1980; Oliver et al., 1983). Circumstantial evidence also suggests that there may be sources of virus other than sheep in SA-MCF as a number of outbreaks have been observed in which no contact with sheep was reported (Straver and van Bakkum, 1979). Domestic goats

and rabbits (*Oryctolagus cuniculus*) have been mentioned as possible reservoirs (Blood et al., 1979). In our study the high seroprevalence (72%) and the positive PCR results detected in the sheep samples, which originate from the same circumscribed area as the antibody-positive fallow deer, might indicate that in this case sheep are the main reservoir animals. However, there is a significant number of outbreaks of cervid MCF in which there has been no obvious contact with carrier animals (Beatson and Hutton, 1981; Denholm and Westbury, 1982). It is possible that deer in these instances were exposed to the supposed virus (OvHV-2) from undefined carriers or that these deer had been latently-infected and developed disease under certain conditions. The evidence that the putative OvHV-2, which apparently naturally circulates in sheep (Baxter et al., 1993; Li et al. 1998), may have a causative role in bovine and cervid MCF is corroborating, as agent-specific DNA has been repeatedly detected in cases of cervid and bovine sheep-associated MCF (Tham, 1997; Tomkins et al., 1997).

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