Caprine Herpesvirus-2–Associated Malignant Catarrhal Fever in White-Tailed Deer (*Odocoileus Virginianus*)

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Caprine herpesvirus-2–associated malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*)

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Abstract. A subacute disease presenting primarily as alopecia and weight loss occurred in 2 white-tailed deer (*Odocoileus virginianus*) on farms in Minnesota and in Texas. A presumptive diagnosis of malignant catarrhal fever (MCF) was made on the basis of histological lesions. Antibody against an epitope conserved among the MCF group viruses was detected in the serum of both deer. DNA samples from the deer were subjected to a variety of PCR amplifications. Alignment of the amplified sequences from the diseased animals revealed that they were 100% identical to each other and to the same DNA fragment from the newly recognized member of the MCF virus group endemic in domestic goats (*Capra hircus*), provisionally named caprine herpesvirus 2 (CpHV-2). A seroprevalence survey from one of the deer farms showed a high rate of subclincal infection in the deer population. This study provides further confirmation that CpHV-2 is a pathogen, at least for deer, and emphasizes the risk of loss from MCF when mixing cervids with goats.

Malignant catarrhal fever (MCF) is a viral disease syndrome primarily of ruminants, caused by a group of closely related rhadinoviruses. The disease is characterized primarily by lymphoproliferation, mucosal inflammation, and vasculitis.[13] Its clinical course ranges from peracute to chronic.[15,19] Of several closely related MCF viruses, only one has been propagated in vitro and partly characterized.[4,18] This virus, endemic in the wildebeest, is known as alcelaphine herpesvirus 1 (AIHV-1). It causes the classic 'African' form of MCF, also known as wildebeest-associated MCF (WA-MCF).[17] The virus known as ovine herpesvirus 2 (OvHV-2) is the major MCF virus worldwide. This virus is endemic in sheep and causes sheep-associated MCF (SA-MCF) outbreaks in various ruminant species. OvHV-2 has never been propagated in vitro.

Recently, 2 additional members of the MCF virus group were reported, both of which were associated with clinical disease. One caused classical MCF in white-tailed deer (*Odocoileus virginianus*)[7]; the reservoir host for this virus has not been identified. The other, endemic in domestic goats (*Capra hircus*), provisionally termed caprine herpesvirus 2 (CpHV-2),[2,8] was associated with chronic alopecia in Sika deer (*Cervus nippon*).[3] This report describes 2 outbreaks of CpHV-2–induced MCF in white-tailed deer.

Case No. 1 occurred on a deer farm in Minnesota. The farm contained more than one hundred animals, including white-tailed deer, Sika deer, fallow deer, pygmy goats (*Capra hircus*), and a variety of nonruminant species. Five white-tailed deer had died the previous year with similar clinical symptoms. A 6-year-old female white-tailed deer was frequently found recumbent over the previous 4-week period. Examination revealed significant weight loss, large areas of alopecia around the mouth and eyes, on both sides of the body, and on the legs. Vision appeared to be impaired. The animal was bled, euthanized, and tissue samples collected at necropsy. Gross skin lesions included severe and widespread alopecia, thickening, crusting, hyperkeratosis, and moderate focal ulceration on the distal extremities. The hoof walls had been shed from the lateral digits on 2 of the feet.

Histopathology revealed lymphoplasmacytic and necrotizing arteritis in the rete mirabile and subcutis, coagulation necrosis in lymph nodes, lymphoplasmacytic meningoencephalomyelitis, myocarditis, and retinitis. The epidermis was hyperplastic and multifocally ulcerated, with intracorneal eosinophilic pustules. The walls of many hair follicles, both superficial and deep dermis, and adnexal glands such as Meibomian glands were infiltrated by eosinophils, lymphocytes, plasma cells, and macrophages. Many follicle lumens were filled with eosinophils and cellular debris. A single intrafollicular demodex mite was found. Multiple infundibuli were obliterated by keratin and bacteria. Rare subcutaneous arteries had marked segmental lymphoplasmacytic and necrotizing arteritis.

Histopathological lesions, particularly in rete mirabile (Fig. 1), spinal cord, and eyes and the vascular changes in the subcutis were compatible with MCF. Culture of selected internal organs including liver, kidney, lungs, and spleen yielded no bacterial growth. Serology was negative for antibodies against epizootic hemorrhagic disease (EHD), bluetongue (BT), adenoviruses, and pseudorabies, and positive for bovine viral

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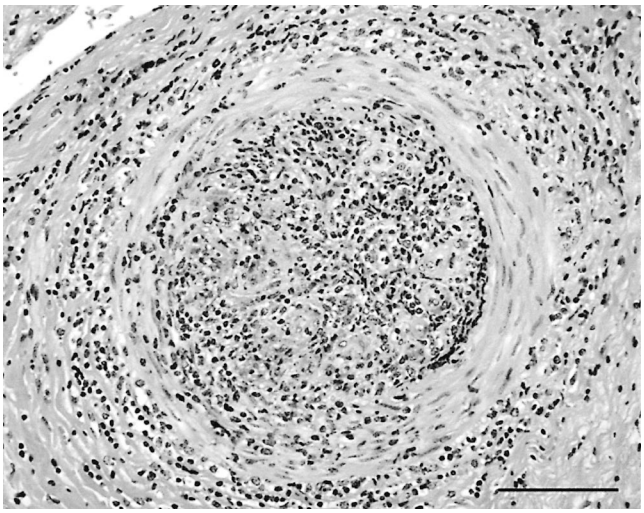

Figure 1. Representative microscopic lesion in an artery of rete mirabile from the diseased white-tailed deer from Minnesota. Note marked lymphoplasmacytic and histiocytic infiltration of the media (segmental) and adventitia and intimal proliferation. Hematoxylin and eosin. Bar = 100 μm.

diarrhea virus (BVDV) antibody. Tests for antibody against the MCF virus group were positive by competitive inhibition ELISA (cELISA).[9] Immunohistochemistry revealed no detectable BVDV antigen in any tissues examined, including skin, lymph nodes, and brain.

Case No. 2 involved a 2-year-old male white-tailed deer on a farm in Texas with 6 does and a herd of various breeds of domestic goats. There were no sheep (*Ovis aries*) in the vicinity. The deer had been losing weight for 2–3 weeks, was mildly lame, and had cutaneous lesions over 1 thigh. At necropsy, the only remarkable gross lesions were enlarged lymph nodes and hyperemia of peritoneal surfaces. The most prominent histologic changes were inflammation and necrosis in multiple large muscular arteries in the mesentery and around lymph nodes. No skin was available for histological examination. This deer was seropositive for the MCF virus group. Because of insufficient serum available, it was not tested for other organisms.

Demonstration of the antibody against the conserved epitope of the MCF virus group in deer from both farms indicated that these deer were infected with 1 or more MCF viruses. To identify which MCF virus was present, 3 polymerase chain reactions (PCRs) were performed, using primers specific for 1) sheep-associated MCF virus, OvHV-2,[11] 2) the MCF virus of unknown origin causing MCF in white-tailed deer (MCFV-WTD),[7] and 3) CpHV-2, the recently described virus endemic in domestic goats.[8] For identity confirmation, consensus PCR was also used to target a conserved region of the herpesviral DNA polymerase gene[21] and a region of the glycoprotein B gene. Amplified DNA fragments were cloned and sequenced.[8] The selection of degenerate primers for a portion of the herpesviral glycoprotein B gene was based on Genbank sequences of AIHV-1, herpesvirus saimiri, human herpesvirus 8, and equine herpesvirus 2. The upstream and downstream primers for the primary amplification were 5′-CARVTNCARTWTGCMTAYGA-3′ and 5′-AGTTRTAYTCYCKRAASAT-3′ and for the secondary amplification were 5′-CVTGGTGYMKNG-ARCA-3′ and 5′-AGTTRTAYTCYCKRAASAT-3′. The amplification conditions were previously described.[8] To avoid error from exogenous DNA contamination, sample DNA was extracted from the various tissues of the affected deer on 3 occasions, simultaneously with MCF virus-negative tissues as controls.

PCR, using primers specific for OvHV-2 or the MCFV-WTD, failed to amplify any DNA fragments from peripheral blood leukocytes (PBL) or the lymph node samples of either white-tailed deer (Fig. 2). However, PCR with primers specific for CpHV-2 yielded a 144-bp DNA fragment (Fig. 2), the specificity of

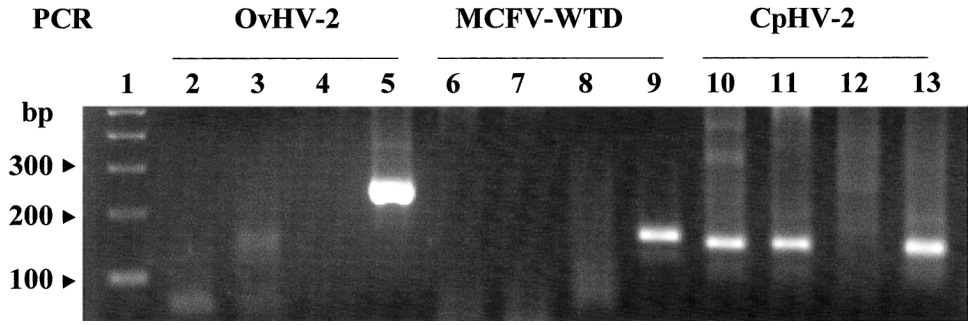

Figure 2. Agarose gel electrophoresis of ethidium bromide–stained PCR products amplified from DNA samples with primers specific for OvHV-2 (lanes 2–5), MCFV-WTD (lanes 6–9), and CpHV-2 (lanes 10–13). Lane 1, 100-bp DNA ladder; lanes 2, 6, and 10, DNA extracted from PBL of the diseased white-tailed deer from Minnesota; lanes 3, 7, and 11, DNA extracted from the lymph node of the diseased white-tailed deer from Texas; lanes 4, 8, and 12, no DNA controls; lanes 5, 9, and 13, positive DNA control from respective hosts (a sheep with OvHV-2; white-tailed deer with MCFV-WTD, and a goat with CpHV-2).

Table 1. Detection of MCF viral antibody and DNA in clinically normal deer and goats by cELISA and PCR.

Species	Farm	Number tested	Number positive by		
			cELISA	OvHV-2 PCR	CpHV-2 PCR
Sika deer	Minnesota	11	3	0	0
White-tailed deer	Minnesota	9	3	0	2
Fallow deer	Minnesota	8	7	0	0
Pygmy goats	Minnesota	6	4	0	3
Unidentified domestic goats	Texas	9	9	0	9

which was confirmed by use of a CpHV-2 probe in dotblot hybridization.[8] The relative levels of viral DNA in PBL or lymph node tissue of both white-tailed deer were evaluated by PCR using endpoint dilution of the samples. The results revealed about 100-fold higher viral DNA levels than levels of the DNA typically found in CpHV-2 carrier goats. The viral DNA levels in the diseased deer were similar to those found in cattle and bison with acute sheep-associated MCF. Using degenerate primers, fragments of 232 bp from the DNA polymerase gene and 580 bp from the glycoprotein B gene were amplified, cloned, and sequenced. The sequences amplified from both diseased deer were 100% identical to the corresponding regions of CpHV-2. PCR did not amplify a fragment from negative tissue controls.

Blood samples were collected from goats at both farms and tested for MCF viral antibody and DNA. As shown in Table 1, 4 of 6 pygmy goats from the Minnesota farm were seropositive and 3 contained detectable CpHV-2 DNA. All 9 domestic goats from the Texas farm were both seropositive and CpHV-2-PCR positive. OvHV-2 DNA was not found in any of the 15 goats examined. The sequence of the DNA polymerase gene fragments amplified from the goats on both farms was 100% identical to CpHV-2, confirming the identity of the virus in the goats.

Examination of sera from clinically normal deer on the Minnesota farm revealed that 3 of 11 Sika deer, 3 of 9 white-tailed deer, and 7 of 8 fallow deer were seropositive. Two of the 3 seropositive, clinically normal white-tailed deer had CpHV-2 DNA in their PBL. No OvHV-2 DNA was detected in any of these 28 normal deer examined (Table 1). The seropositivity rate of the deer was higher than expected, as previous reports of prevalence rates ranged only up to 16% in free-ranging and captive deer.[5,10,12] The explanation for the high rate on this farm is not clear, although a recent improvement in the sensitivity of the MCF cELISA[9] may be partly responsible. The fact that 2 clinically normal white-tailed deer from the Minnesota farm contained CpHV-2 DNA in their PBL indicates that CpHV-2 can induce subclinical or latent infection in clinically susceptible hosts, as does OvHV-2. Failure to detect CpHV-2 DNA in other antibody-positive deer is probably a reflection of low copy numbers of viral DNA in peripheral blood. This phenomenon has been observed in other species, such as bison.[16]

Collectively, the data from serology, PCR, sequence analysis, and histopathology reveal an association between the disease in the deer and CpHV-2. Chronic alopecia and dermatitis in 2 Sika deer that were associated with CpHV-2 were reported earlier.[3] The Sika deer experienced a more protracted course than the present cases and had distinct granulomatous mural folliculitis and epidermitis with giant cells and macrophages predominating. The skin contained only widely scattered vascular lesions, but inflammation of the hair follicles was preeminent. In these cases, no Demodex were found and eosinophils were not prominent.

The basic nature of the presentation, chronic weight loss and alopecia, was consistent between the cases in both species (Sika and white-tailed deer). In Case No. 1 of this report, which was the most thoroughly studied of the 2 cases, the presence of Demodex complicates comparison between the 2 reports. The granulomatous character of the skin infiltrates was prominent in the previous report on Sika deer. Determination of how much of the histological difference between the lesions in the previous and the present reports was due to superimposed parasite-induced acute changes will require examination of more cases.

The differences in the skin lesions between the CpHV-2–associated MCF and the sheep-associated virus are also yet to be clearly defined. However, the main differences so far seem to reside in the more prominent vasculitis, lack of mural follicular involvement, and a more acute, less granulomatous character of the cellular infiltrate in typical MCF dermal lesions seen in apparently OvHV-2–induced cases.[20] The degree of clinical susceptibility of the host could be a key factor. As observed by Berkman[1] in a report on bovine MCF, the general prominence of dermal lesions seems to be related to the length of the clinical course, being more marked in the more chronic cases.

Internal organ lesions in both white-tailed and Sika deer were more typical of classical MCF lesions: prominent vasculitis and lymphocytic proliferation, and infiltration. The relatively minor skin lesion differences seen between white-tailed deer and Sika deer infected with CpHV-2 could reflect either species-dependent susceptibility or virulence differences between as-yet-unidentified strains of CpHV-2. Variation in susceptibility to OvHV-2 has been reported among cervid species.[19] As yet, there is no information about

the possible existence of substrains within the goat-associated MCF virus.

The primarily dermatotropic clinical presentations in this report and in the previous report on Sika deer[3] are a variation on the typical MCF disease pattern. The body of MCF literature, however, contains descriptions of many different facets of the disease, with a diverse array of predominant organ involvement and lesion types. The protean nature of disease expression by gammaherpesvirus infections is consistent with their possession of multiple regulatory genes, acquired from host cells during evolution.[14] The selection and degree of expression of these genes may well vary from host cell to host cell, as well as between animal species, providing an ample basis for multiple disease expressions.

The precise relationship between the virus and the skin lesions in these cases is yet to be defined. However, the clear association, for example, between Epstein–Barr virus and emerging syndromes in humans characterized by granulomatous dermatitis with folliculitis[6] illustrates the richness of disease expression, including dermatotropic presentations, of which this group of viruses is capable.

Inability to detect OvHV-2 by PCR in seropositive animals with apparent clinical MCF has led to considerable confusion in the past. Recognition of CpHV-2 and its pathogenicity for deer is an important step toward clarifying such diagnostic confusion. MCF is a serious problem for zoos, exotic game farms, and other mixed-ruminant species operations. Although MCF losses due to CpHV-2 can be avoided by separation of infected goats from clinically susceptible species, production of CpHV-2-free goats is a better control alternative. A study of transmission of CpHV-2 among goats is underway, which should define the parameters on which to base a program for production of CpHV-2–free goats.

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