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## Epidemiologic and Pathologic Aspects of an Epizootic of Malignant Catarrhal Fever in Exotic Hoofstock

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**ABSTRACT:** An epizootic of malignant catarrhal fever (MCF) occurred at the Los Angeles Zoological Park which resulted in the deaths of four exotic ungulates. The source of infection was considered to be a newly purchased wildebeest bull (*Connochaetes taurinus taurinus*) that had been negative for antibody to MCF virus by an indirect immunofluorescent test. The need to re-evaluate regulations for the transportation and housing of young wildebeest is emphasized by this MCF outbreak. The diagnostic technology now available for identifying asymptomatic carriers of MCF virus and the present understanding of the behavior and pathogenesis of this highly cell-associated herpesvirus in exotic ruminants should provide a basis for the prevention and control of MCF in zoological parks.

**Key words:** Malignant catarrhal fever, *Connochaetes taurinus taurinus*, virology, alcelaphine herpesvirus-1, epizootic, captive study.

In November 1984, three healthy Chinese water deer (*Hydropotes inermis*) and a stone sheep (*Ovis dalli stonei*) were imported from Canada to the Los Angeles Zoological Park (Los Angeles, California 90027, USA) and placed on informal quarantine but were exhibited. The enclosure housing the deer was located at the bottom of a hill along a road that drained four contiguous uphill pens. At the top of this drainage system was the wildebeest (*Connochaetes taurinus taurinus*) exhibit. The sheep was delivered elsewhere and its placement was uncertain.

Two wk after arrival, one of the Chinese water deer appeared weak, became recumbent and died within 5 hr. A second doe developed diarrhea 2 wk later and died after a rapid course of illness which progressed to ataxia and weakness. Located about 100 m from the quarantine pen which housed the water deer were the ger-

enuk (*Litocranius walleri*) and red flanked duiker (*Cephalophus rufilatus*) enclosures. One death occurred in each of these exhibits (Fig. 1). All deaths occurred over a 2-mo period; however, none of the affected animals was clinically ill longer than 12 hr.

Necropsy findings in each of these cases were few and nonspecific. A lesion consistently seen in all cases was uniformly firm, deep red to purple lungs. A hemorrhagic enterocolitis was present in one Chinese water deer. The duiker had enlarged cervical and mandibular lymph nodes and small tan spots were present throughout the liver. The scarcity of lesions seen in cases of malignant catarrhal fever (MCF) in exotic hoofstock is thought to be a function of the rapid and severe course of the

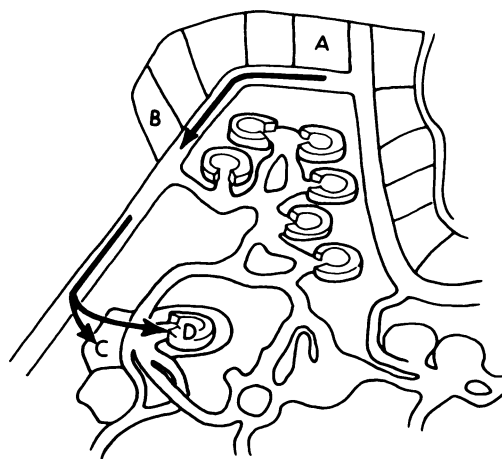


FIGURE 1. Pattern of malignant catarrhal fever occurrence in the Los Angeles Zoo; subscripts represent areas where wildebeest (A), Chinese water deer (B), gerenuk (C), and red flanked duiker (D) were housed.

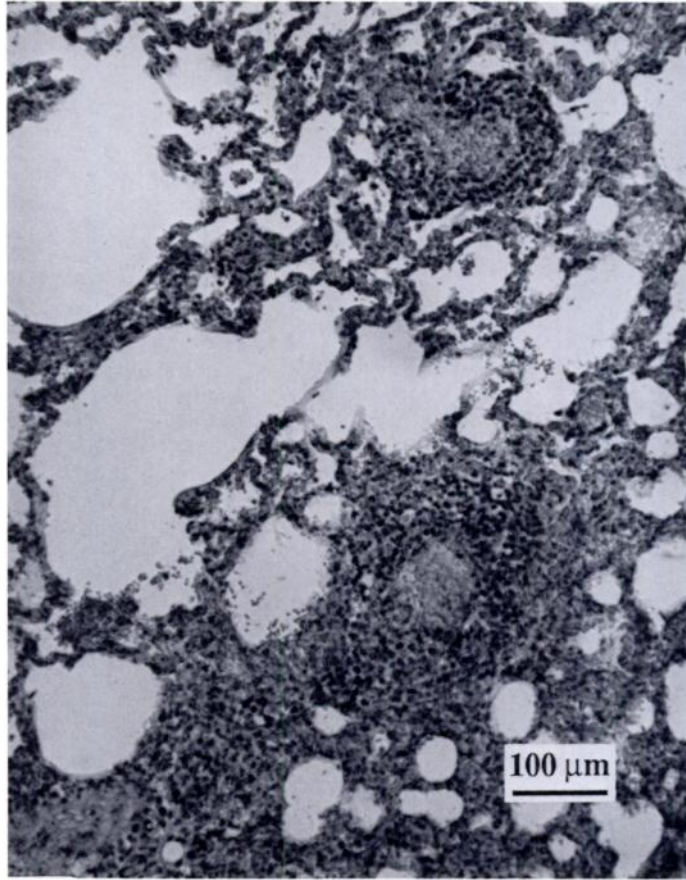


FIGURE 2. Dense lymphocytic perivascular cuffs and vasculitis of pulmonary vessels in a Chinese water deer with MCF. H&E.

disease in these species (Zimmer et al., 1981; Heuschele et al., 1984b).

A provisional diagnosis of MCF was based on clinical and histologic findings. Tissues were taken at necropsy and fixed in 10% buffered formalin, embedded in paraffin, cut to 5- $\mu$ m sections and stained with hematoxylin and eosin. Examination of tissues by light microscopy revealed generalized lymphocytic vasculitis in the liver, lung, kidney, brain, meninges, adrenal cortex and the limbus of the eye. An infiltrate of lymphocytes and lymphoblasts was seen within the adventitia of medium and small arteries and veins. These lymphocytic aggregates formed dense perivascular cuffs which were especially prominent around the renal arcuate ves-

sels, the portal areas of the liver and pulmonary vessels (Figs. 2, 3). Similar lymphoproliferative lesions have been described in cases of MCF (Liggitt and DeMartini, 1980; Zimmer et al., 1981; Heuschele et al., 1984b). In a recent case report, lymphocytic infiltrates in the intestine of a sika deer (*Cervus nippon taiouanus*) were so severe that they were diagnosed as lymphoma (Heuschele et al., 1985).

Attempts were made to isolate and identify MCF virus from the tissues of the water deer by methods previously reported (Heuschele, 1983; Heuschele et al., 1985) using 10 serial blind passages of tissue extracts in fetal aoudad kidney cell cultures. These were unsuccessful. Our inability to

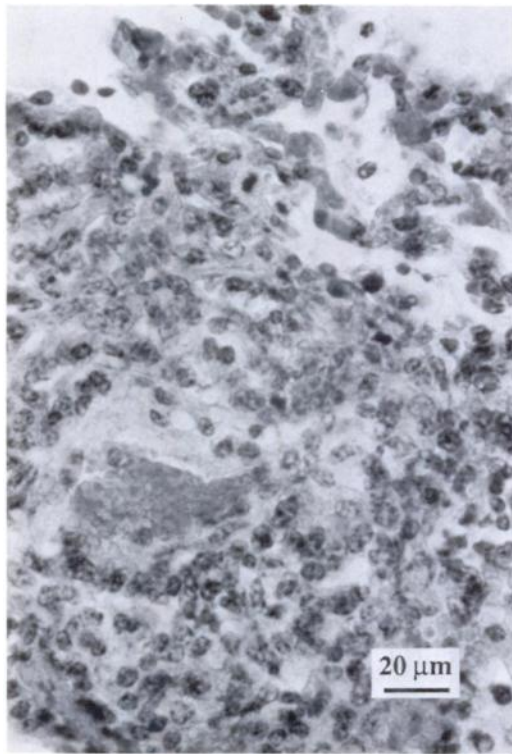


FIGURE 3. Lymphocytic vasculitis and perivasculitis in the lung of a Chinese water deer. Note the mitoses. H&E.

isolate this virus was probably due to its temperature sensitivity and its rapid inactivation after the animal's death (Heuschele and Fletcher, 1984).

Sera were not available to determine the antibody status of each MCF affected animal prior to the outbreak. Sera were obtained from some animals during their clinical illness. These were tested for MCF antibodies by indirect immunofluorescence (IIF) and serum virus neutralization (SVN) by methods previously described (Heuschele, 1983; Heuschele and Fletcher, 1984). Results of serologic tests for MCF performed after the resolution of the outbreak at the Los Angeles Zoo demonstrated a suspect positive IIF test for MCF in the second water deer to die. This animal was seronegative by SVN. The wildebeest calf and its sire, penned near the water deer, were seropositive to MCF virus by SVN

tests. The third healthy Chinese water deer was seropositive by SVN as were six of 17 Reeve's muntjacs (*Muntiacus reevesi*) and nine of 114 randomly tested hoofstock (Table 1). Retrospectively, the clinical, histological and serologic data supported a diagnosis of MCF due to alcelaphine herpesvirus-1.

Alcelaphine herpesvirus-1 belongs to the subfamily Gammaherpesvirinae. This group of herpesviruses are highly cell-associated and are usually carried as latent nonclinical infections in their reservoir hosts. Infectious, cell-free alcelaphine herpesvirus-1 has been found in nasal and ocular secretions in healthy wildebeest up to 3-mo-old and adult wildebeest after stress or corticosteroid therapy (Mushi et al., 1981; Heuschele and Fletcher, 1984; Heuschele et al., 1984b). Naturally occurring cases of MCF in domestic cattle and many species of exotic hoofstock have been associated with apparently healthy lambing sheep and calving Bovidae of the subfamily Alcelaphinae, which include wildebeest, hartebeest (*Alcelaphus buselaphus cokei*) and topi (*Damaliscus korrigum*) (Pierson et al., 1979). Whether MCF is contracted from sheep or wildebeest, the clinical disease is characterized by sporadic morbidity but high mortality (Pierson et al., 1973) and the histopathologic lesions of MCF are similar (Pierson et al., 1979). Both sheep and alcelaphine-associated MCF have been transmitted with infectious blood but only the alcelaphine MCF virus has been isolated (Castro et al., 1982; Heuschele and Castro, 1984; Reid et al., 1984) and it is assumed that the viruses indicted in the sheep and alcelaphine-associated MCF are closely related but not identical (Rossiter, 1981; Heuschele and Fletcher, 1984; Heuschele et al., 1984b).

Wildebeest calving had not occurred at the Los Angeles Zoo in the 5 mo prior to the arrival of the Chinese water deer. The 5-mo-old wildebeest appeared healthy and was older than calves in previously documented cases of spontaneous cell-free virus

TABLE 1. Serum virus neutralization and indirect immunofluorescent antibody results for antibody to MCF in exotic ungulates at the Los Angeles Zoo.

Species		IIF <sup>a</sup>	SVN <sup>b</sup>
<i>Addax nasomaculatus</i>	Addax	— <sup>c</sup>	1:32
<i>Capra ibex nubiana</i>	Nubian ibex	—	1:8
<i>Capra falconeri</i>	Turkomen markhor	—	1:6
<i>Cephalophus zebra</i>	Zebra duiker	Suspect at 1:20	1:2
<i>Connochaetes taurinus taurinus</i>	Brindled gnu (bull)	—	1:16
<i>C. taurinus taurinus</i>	Brindled gnu (calf)	—	1:4
<i>C. taurinus taurinus</i>	Brindled gnu	1:20	ND <sup>d</sup>
<i>Hippotragus niger</i>	Sable antelope	—	1:8
<i>Hydropotes inermis</i>	Chinese water deer	1:20	—
<i>H. inermis</i>	Chinese water deer	—	1:8
<i>Kobus megaceros</i>	Nile lechwe	1:20	—
<i>K. megaceros</i>	Nile lechwe	1:100	—
<i>Lama pacos</i>	Alpaca	—	1:6
<i>Muntiacus reevesi</i>	Reeve's muntjac	—	1:6
<i>M. reevesi</i>	Reeve's muntjac	—	1:6
<i>M. reevesi</i>	Reeve's muntjac	—	1:8
<i>M. reevesi</i>	Reeve's muntjac	—	1:8
<i>M. reevesi</i>	Reeve's muntjac	—	1:12
<i>M. reevesi</i>	Reeve's muntjac	—	1:64
<i>Oryx gazella dammah</i>	Scimitar-horned oryx	—	1:32
<i>Oryx leucoryx</i>	Arabian oryx	1:20	ND
<i>Ovis dalli</i>	Dall's sheep	1:20	1:8
<i>Ovis vignei</i>	Turkomen urial	—	1:16
<i>Tragelaphus euryceros</i>	Bongo	1:20	—

<sup>a</sup> Indirect immunofluorescent antibody.

<sup>b</sup> Serum virus neutralization.

<sup>c</sup> Negative at 1:20.

<sup>d</sup> Not done.

shedding (Mushi et al., 1981; Castro et al., 1984). The wildebeest herd was not under treatment for illness or receiving corticosteroid therapy. Unusual stressful environmental factors could not be identified. Therefore, a reason for the release of cell-free virus could not be determined. Nevertheless, transmission of the virus was believed to have occurred via the drainage route that passed the pen housing the Chinese water deer. From this point, the gerenuk and red flanked duikers may have been contaminated through a common feeding and service shed across the road from the water deer. The arrows in Figure 1 illustrate the service road that accesses the duiker and gerenuk exhibit and this also may have been a route of contamination.

A similar epidemiological problem occurred at the Oklahoma City Zoo in 1979 (Zimmer et al., 1981) resulting in deaths in a herd of Indian gaur (*Bos gaurus*) that were housed near the base of a drainage route that included a herd of wildebeest upstream. However, cows in this wildebeest herd were calving prior to, and during, the clinical manifestations of MCF which resulted in the death of three Indian gaurs and a greater kudu (*Tragelaphus strepsiceros*). The etiology of this outbreak was later confirmed by the isolation of an alcelaphine herpesvirus in cell cultures, fluorescent antibody tests, electron microscopy and animal inoculation (Castro et al., 1982, 1984).

The wildebeest herd at the Los Angeles Zoo was established with two cows and a

bull imported from Africa. All were MCF seronegative through 1982 as determined by a series of IIF tests. Nineteen offspring were produced by the original breeding stock until the male died of coccidioidomycosis in 1978. No new introductions of wildebeest were made for 5 yr and there had been no known clinical cases of MCF associated with this herd.

In April 1983, a new breeding male was obtained from a privately owned wild animal park. This animal tested negative for MCF by IIF and was introduced into the established herd. Two calves, sired by this bull, were born the following year. One calf was euthanized when 5-mo-old because of cataracts and trauma. A necropsy was performed and there was no evidence of infectious disease. The second calf was 5-mo-old when the Chinese water deer arrived at the zoo in November of 1984.

Routinely, all animals that die at the Los Angeles Zoo are necropsied. Thus, a retrospective histological evaluation was performed on hoofstock that died between January 1984 and February 1985. Histological evidence of MCF was not found in any tissues sampled from the 40 hoofstock necropsied during the year prior to the arrival of the Chinese water deer. The wildebeest were removed from the Los Angeles Zoo and there have been no further cases of MCF during the subsequent 15 mo. The third Chinese water deer remains healthy and there have been no reported problems with other members of the original Canadian herd.

Although epidemiologic data indicate that domestic cattle are dead-end hosts of MCF (Plowright, 1968), recent reports and serologic data suggest the potential for certain wild sheep and goat species, as well as domestic sheep (Heuschele and Castro, 1984), to act as reservoir hosts for MCF. Deer are also being considered as potential reservoirs for MCF. There have been reports of the successful isolations of cell-free alcelaphine herpesvirus-1 from feces and nasal-ocular secretions of a clinically ill sika

deer fawn and buffy coat cells of experimentally infected white-tailed deer (*Odocoileus virginianus*) (Whitenack and Castro, 1981; Heuschele et al., 1985). Rabbits are highly susceptible to both alcelaphine and sheep-associated MCF. Sixteen brush rabbits from the wildebeest and hartebeest habitat at the San Diego Wild Animal Park were tested for MCF antibody by SVN and two were found to have low titers (Heuschele et al., 1984a). It has not been determined whether all seropositive animals are capable of shedding the cell-free virus that could potentially infect domestic and exotic hoofstock. It is assumed, however, that animals positive for SVN antibodies are probably carriers of the viral genome (Heuschele et al., 1984a; Castro et al., 1985).

Serum virus neutralization tests appear to be most specific for antibodies to alcelaphine MCF (Heuschele et al., 1984a). Cross reactions of antibodies to other bovine herpes viruses have been demonstrated by IIF with alcelaphine MCF virus antigen (Heuschele et al., 1984b). Due to this lack of specificity, it has been recommended that the IIF test be discontinued as a diagnostic test for MCF (Castro et al., 1985). Recently, Wan et al. (1988) have shown that exotic ruminants with sheep or alcelaphine associated disease had antibodies to alcelaphine herpesvirus-1 as measured by an enzyme-linked immunosorbent assay (ELISA).

Recommendations have been proposed to reduce the potential spread of MCF<sup>6</sup> (Ramsay et al., 1982). These policies would allow movement of alcelaphine antelope between zoos or parks based on negative ELISA and/or SVN tests. These serologic assays would also be used to help direct the housing of susceptible hoofstock, particularly Asiatic species, in relation to the location of alcelaphine herds. A policy to prohibit any alcelaphine antelope from private game ranches that could potentially expose domestic cattle and wildlife to MCF has also been proposed in these

recommendations.<sup>6</sup> This restriction of alcelaphine species from game ranches seems reasonable due to the lack of uniformity of testing used for screening of exotic species and in light of this case report in which apparent spread of MCF was traced to a wildebeest that was clinically normal and IIF negative as well as a report of the isolation of alcelaphine herpesvirus-1 from a wildebeest calf with no demonstrable MCF antibodies (Castro et al., 1984).

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