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EXPERIMENTAL MALIGNANT CATARRHAL FEVER (AFRICAN FORM) IN WHITE-TAILED DEER[1]

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Abstract: White-tailed deer *(Odocoileus virginianus)* were experimentally infected with the African form of malignant catarrhal fever (AMCF) virus by inoculation of whole blood from experimentally infected cattle, from whole blood obtained from a greater kudu *(Tragelaphus strepsiceros)* and from virus isolated in cell culture. The incubation period from AMCF in experimentally infected deer ranged from 13 to 18 days. Clinical disease was characterized by lacrimation, an elevated body temperature, conjunctivitis and swelling of the external lymph nodes. Histologic lesions were primarily characterized by widespread vasculitis and lymphadenopathy. The organs most severely affected were liver, lymphoid tissue, brain and lungs. Successful recovery and identification of AMCF virus was accomplished from one experimentally infected deer.

INTRODUCTION

Malignant catarrhal fever (MCF) is an infectious, usually fatal viral disease of ruminants. Two forms of the disease have been recognized. In Africa, a wildebeest-cattle associated disease has been observed for more than a century. Wildebeest become inapparently infected during the calving period and transmit the disease to buffalo and cattle. The etiologic agent of this disease has been identified and characterized as a herpesvirus.[9] The method of natural transmission is unknown. Direct contact by nasal secretions however, has been proposed as a method of virus transmission among wildebeest and from wildebeest to cattle.[11]

In other areas of the world, including the United States, MCF has been proposed as a sheep-cattle associated disease. A viral candidate for the American form of MCF has been identified recently[5] although other viral particles, identified morphologically, also have been implicated.[4] Diagnosis in all cases has been based on the clinical disease profile and histopathologic findings.[3,5,6,7,8,12,13]

This report presents the first evidence for the transmissibility, pathogenicity and clinical and histopathologic changes observed in white-tailed deer *(Odocoileus virginianus)* experimentally infected with the African form of MCF virus.

MATERIALS AND METHODS

Following the isolation, characterization and successful cultivation of the African form of MCF virus (AMCFV) which was originally observed during a naturally occurring outbreak at the Oklahoma City Zoo,[14] experimental studies were initiated to determine the

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susceptibility, pathogenicity and the course of the clinical disease in experimentally infected white-tailed deer.

Confirmation of AMCFV was done by animal inoculation, isolation of an agent in bovine embryonic kidney (BEK) cultures with typical cytopathic effects (CPE) of MCF[1,0] (Figs 1 and 2), electron microscopy[1,2] (Fig 3) and, in one case, specific reactivity by the fluorescent antibody test with immune serum prepared against the AMCFV in bovines.[2]

Four adult white-tailed deer were used to determine the susceptibility of this species to AMCFV. All infections were initiated by intravenous inoculation with either heparinized whole blood or virus propagated in cell culture. Diagram 1 illustrates the infection schedule for the four deer.

Deer no. 1 was inoculated intravenously (IV) with 50 ml of whole blood from the experimentally infected Holstein bull calf (Bovine no. 2). At the time of the deer inoculation, Bovine no. 2 was in the 21st day after inoculation with blood from the Angus-Hereford yearling heifer (Bovine no. 1). The temperature of bovine no. 2 at

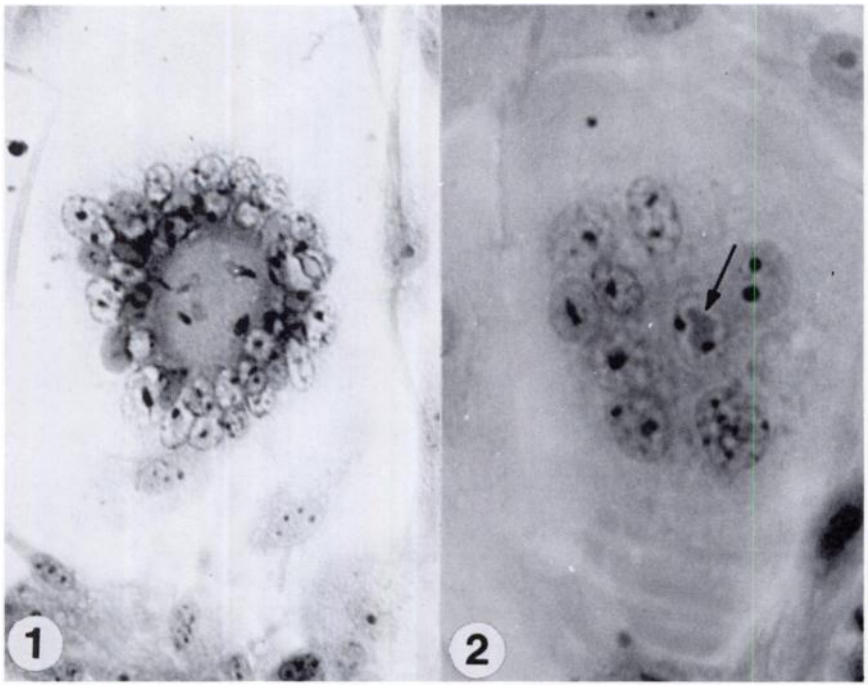

FIGURE 1. Cellular cytopathology produced by herpesvirus isolated from white-tailed deer by co-cultivation of AMCF-infected bovine embryonic kidney with bovine thyroid cells. Syncytium produced in 4 to 7 days (H & E stain, X 200).

FIGURE 2. Syncytium produced by AMCFV in bovine embryonic kidney cells. Note intranuclear inclusion body in central cell (arrow) (H & E stain, × 400).

[2] Antiserum was obtained courtesy of Dr. J. Callis, USDA, Plum Island Animal Disease Center, Greenport, New York 11944, USA.

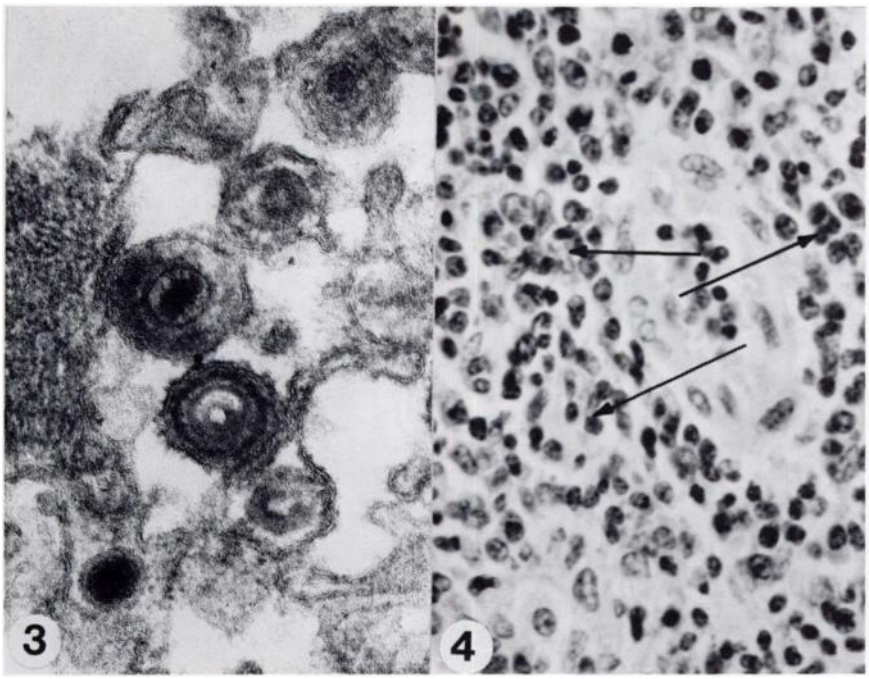

FIGURE 3. Electron micrograph of a thin section of bovine embryonic kidney cell with enveloped nucleocapsids of herpes virions (129 nm) located in cytoplasm. Virus was isolated from AMCF-infected white-tailed deer. Sections were stained with uranyl acetate and lead citrate (× 116,000).

FIGURE 4. Accumulation of pleomorphic lymphoid cells (arrows) within a portal triad of the liver. (H & E stain × 400).

that time was 41.2 C. Clinically, a conjunctivitis and nasal discharge was seen. A second deer (no. 2) was placed in the same isolation room with deer no. 1 but was not inoculated.

Deer no. 3 received a 20 ml IV inoculation of AMCFV ($10^{4.45}$ $TCID_{50}$/ml) grown in BEK which was isolated from the heifer calf (originally inoculated (IV) with whole blood obtained from a Gaur). Infected cultures containing disrupted cells from the third and fourth virus passage were used as the inoculum.

A simultaneous inoculation of 20 ml of cell culture virus was given IV to deer no. 2 (originally the uninfected control housed with deer no. 1) at 28 days after deer no. 1 had shown clinical signs of disease. Prior to inoculation, no indication of clinical disease was noted in deer no. 2, and CPE was not observed in BEK cells co-cultivated with buffy coat extracts. Procedures for cell culture isolation and maintenance are described elsewhere.[1,2]

Deer no. 4 was given a 50 ml IV inoculation of whole blood obtained from a Greater Kudu which had died at the Oklahoma City Zoo with signs of disease resembling MCF. Strict isolation procedures were exercised for all experimental animals. Following evaluation all carcasses and all culture material was incinerated.

RESULTS

Deer no. 1. The first sign of disease noted in this animal was unilateral lacrimation 16 days after exposure. On day 17 after exposure, the temperature of the deer was 41.8 C. At this time, the deer had bilateral lacrimation and the conjunctiva were reddened and swollen. Scleral vessels were congested and very prominent. There was excessive salivation and the oral mucosa was reddened. Neither nasal exudate nor erosions of the oral cavity were observed. The deer was slightly depressed but alert and still drinking and eating at 17 days after exposure. At 18 days after exposure the deer was depressed, respiration was rapid and the animal was weak and uncoordinated. The right eye had a mucous exudate at the lateral canthus. Rectal temperature was 41.1 C. The animal died during the night of the 18th day after exposure.

At necropsy, the nasal mucosa was reddened but no exudate was observed. The tracheal mucosa was reddened and the lumen contained a hemorrhagic froth. The lungs were diffusely reddened and had generalized petechiations scattered throughout the parenchyma. The myocardium was pale and there was endocardial hemorrhage on the left ventricle just below the valves. The capsular surfaces of the kidneys had numerous small white foci on their surfaces. The spleen was enlarged and friable. Peripheral lymph nodes were enlarged and edematous. Fat around lymph nodes was hemorrhagic.

Evidence of AMCFV was based on typical CPE produced in BEK cells and histopathologic changes.

Deer no. 2. This deer was the asymptomatic contact control which was housed with deer no. 1. It remained asymptomatic for 28 days after deer no. 1 died.

At 15 days after inoculation with 20 ml of virus ($10^{3.45}$ $TCID_{50}$/ml) propagated in cell culture, deer no. 2 became depressed. Upon physical examination, the prescapular and submandibular lymph nodes were enlarged and the nostrils were swollen. The rectal temperature was 41.7 C. At 16 days after inoculation the deer was euthanatized. Five hundred ml of blood which was defibrinated with glass beads was collected for virus isolation.

The gross necropsy changes were similar to those seen in deer no. 1. All external and internal lymph nodes were enlarged and on cut surface were edematous. There were numerous small white foci on the surface of the liver and on the cut surface of the liver parenchyma. The capsular surface of the kidney also had small white foci similar to those seen on the liver. MCFV was diagnosed by CPE produced by lymphoid tissue, spleen, buffy coat cells cocultivated with BEK cells and histopathology.

Deer no. 3. The deer received 20 ml of MCFV ($10^{3.45}$ $TCID_{50}$/ml) propagated in cell culture originally isolated from Bovine no. 1. On day 15 after exposure, the deer showed signs of depression and inactivity. On day 16, the deer became inactive and did not rise. There was a slight serous nasal exudate. At 17 days after infection, the deer had a rectal temperature of 40.7 C and appeared more depressed and was reluctant to move. The deer was killed and tissues and 500 ml of blood were taken for virus isolation.

Gross necropsy changes were comparable with those seen in the other two deer. All internal and external lymph nodes were enlarged and edematous on cut surfaces. The liver and kidneys had generalized pale foci on the capsular and cut surfaces. Confirmation of MCFV was based on CPE in BEK cells infected with buffy coat cells, reactivity by specific fluorescence to AMCFV antibody, and by observation with electron microscopy of a herpesvirus isolated in tissue culture.

Deer no. 4. This deer was inoculated with 50 ml of blood from a Greater Kudu

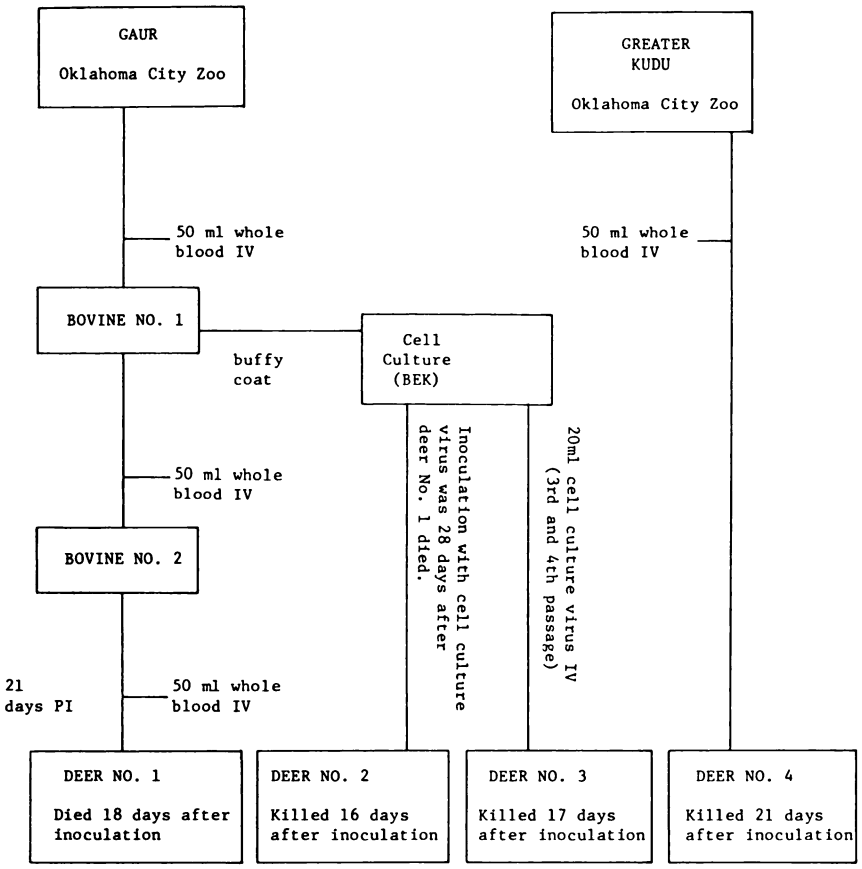

DIAGRAM 1. Infection schedule for captive white-tailed deer with the African form of malignant catarrhal fever virus.

which had died with signs of disease resembling MCF.

At 18 days after exposure, deer no. 4 became inactive and had bilateral mucopurulent exudate from the eyes. At 21 days after infection, the deer was less active, had a slight discharge from both nostrils and eyes, and the rectal temperature was 40.7 C. The deer was euthanatized and blood and tissues were collected for virus isolation.

Necropsy findings revealed changes similar to those seen in the other three deer, a generalized lymphadenopathy and hepatitis suggestive of MCF. MCFV was diagnosed by CPE in BEK cells infected with buffy coat cells and by histopathology.

HISTOPATHOLOGY

Histopathologic lesions in all deer were similar. The lesions were widespread, involving many organs, and were associated with blood vessels. The organs most severely affected were liver, lymphoid tissue, brain and lungs.

Liver. There was a generalized and marked lymphoid cell accumulation in the portal triads. The lymphoid cell infiltrate was multifocal in liver lobules. Central veins were not affected. There was a subcapsular infiltrate of similar cells. The mononuclear cells were pleomorphic, having a large, vesicular nucleus, a prominent nucleolus and moderately abundant cytoplasm. Mitotic figures were common in lymphoid cells. The walls of the blood vessels in portal triads had accumulations of lymphoid cells (Fig. 4).

Lungs. A lymphoid cell accumulation in alveolar walls, medium sized blood vessels and peribronchiolar were the primary lesion in the lungs. The walls of the medium sized vessels were replaced by a coagulum of homogenous eosinophilic, amorphous material containing embedded nuclei. Neutrophils were rare. Morphologically, the primary changes in the lungs were diffuse vasculitis and interstitial pneumonia (Fig. 5).

Lymph nodes. In all lymph nodes examined, including the prescapular, prefemoral, mediastinal, retropharyngeal, hepatic and mesenteric, large lymphocytes were few, being replaced by lymphoreticular cells. Germinal centers were replaced by accumulations of lymphoid cells and eosinophilic fibrinoid material. The medullary sinuses contained lymphoreticular cells (Fig. 6).

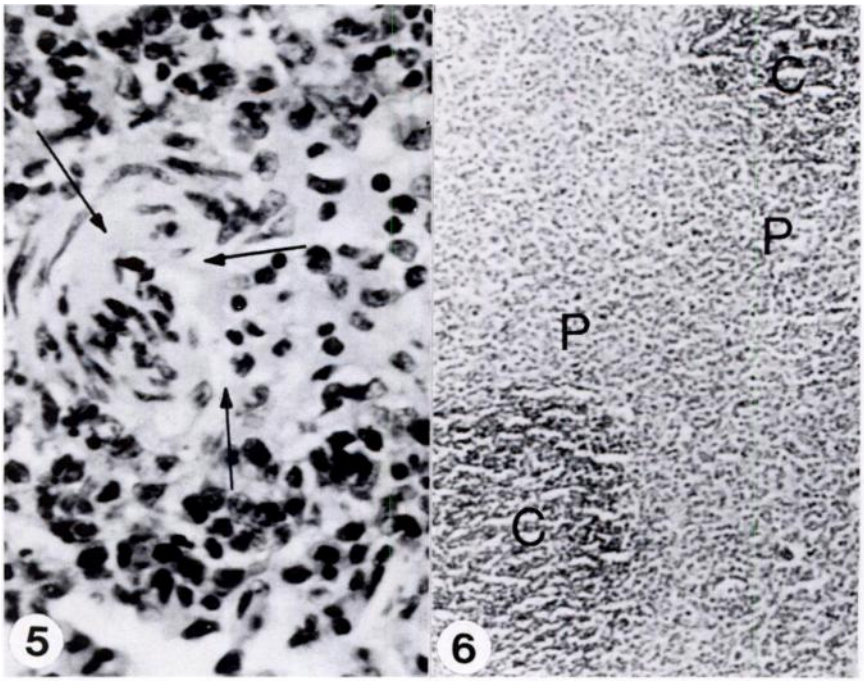

FIGURE 5. Artery in the lungs of a white-tailed deer, experimentally infected with AMCFV. Disruption of vessel wall (arrows), infiltrate of lymphoid cells and eosinophilic coagulum in artery wall is evident (H & E stain × 400).

FIGURE 6. Extensive paracortical lymphoid cell hyperplasia of lymph node (P = paracortical area, C = cortical area). (H & E stain × 100).

Similar cellular infiltrates were seen around and within walls of blood vessels.

Central nervous system. There were generalized lesions in the brains of all deer. These lesions were confined to blood vessels, meninges and choroid plexus. The brain parenchyma had no visible lesions. Distribution of vascular lesions was similar in white and gray matter. The basic lesion was a mononuclear cell infiltrate in the walls and adventitial tissues of blood vessels. In arteries, the walls had fibrinoid degeneration in which reticular cells were embedded. Perivascular infiltrate of cells was minimal (Fig. 7). The choroid plexus of the lateral and fourth ventricles and cerebellum were diffusely affected having marked proliferation of lymphoid cells in the supporting stroma (Fig. 8).

Vasculitis with infiltration by lymphoid cells was the basic lesion observed in other tissues including testes, skin, tongue, buccal mucosa, nasal epithelium, kidney, spleen, pancreas and conjunctiva of the eyes.

DISCUSSION

Reports of the presence of MCF in North American white-tailed deer are scarce. In the United States, the sheep associated form (American) has been reported in white-tailed deer and axis deer in Texas,[4] white-tailed deer in New Jersey and Connecticut,[14] mule deer in Colorado,[8] and sika deer in Ontario.[11] In all instances, diagnosis of the disease has been based on the histopathologic lesions since no etiologic agent has yet been isolated.

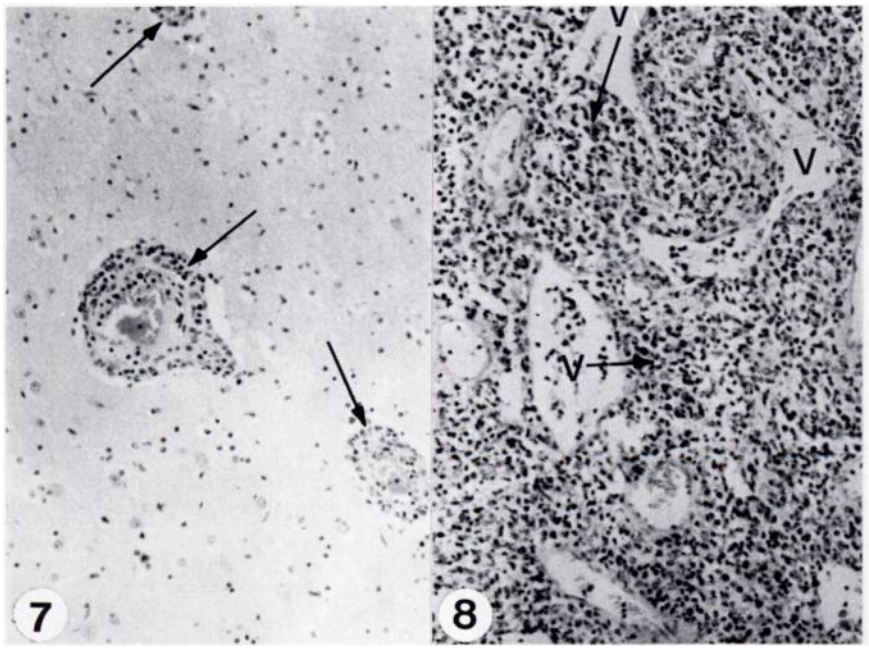

FIGURE 7. Infiltrate of lymphoid cells (arrows) in blood vessels of the cerebrum (V = vessels), (H & E stain × 200).

FIGURE 8. Diffuse lymphocytic cell accumulation (arrows) in choroid plexus of the lateral ventricle (H & E stain × 200).

In the present report, the African form of MCF virus was isolated from a naturally-occurring outbreak in a herd of Gaurs and in a Greater Kudu in a zoo and experimentally transmitted to captive white-tailed deer from experimentally infected calves, by infected cell cultures or parental inoculation of blood. The incubation period ranged from 13 to 18 days with death imminent by the eighteenth day. Infections were initiated by inoculation of 50 ml of whole blood and 20 ml of tissue culture-derived virus. Clinical and histopathologic lesions in the white-tailed deer in this study, which were inoculated with the African form of AMCF virus, were indistinguishable from those reported in other deer in North America presumably infected with the sheep associated form of MCF.[3,11,12] Clinically, the disease is characterized by lacrimation, elevated body temperature, reddening of the eyes and swelling of the external lymph nodes. Erosions of the oral cavity were not evident.

Histopathologic lesions were characterized by a widespread vasculitis and lymphadenopathy. The organs most severely affected were liver, lymphoid tissue, brain and lungs.

The results of this study indicate that white-tailed deer are susceptible to the African form of MCF. Because of the short incubation period observed followed by acute onset of clinical signs and the 100% mortality observed in the four deer in this study, we believe that white-tailed deer serve as a susceptible host and are not latent carriers. It also appears that infection by contact is not a primary method of transmission since our uninfected control deer placed in the same pen as an inoculated deer did not show clinical sign of disease, and we were unable to culture virus from this animal 28 days after its pen-mate had died. This animal did, however, later prove to be susceptible by inoculation with cell culture virus. These findings indicate that the AMCF virus can be maintained in domestic and/or native wildlife species posing a potential threat to these animals where contact with exotic species is possible.

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LITERATURE CITED

1. CASTRO, A.E. and G.G. DALEY. 1981. An electron microscopic study of the African strain of malignant catarrhal fever virus in bovine cell cultures. Am. J. Vet. Res. (Submitted).
2. ——, ——, M.A. ZIMMER, D.L. WHITENACK and J. JENSEN. 1981. Malignant catarrhal fever in *Bos guarus* in Oklahoma: Experimental transmission, isolation and identification of a herpesvirus. J. Am. vet. med. Ass. (Submitted).
3. CLARK, K.A. and R.M. ROBINSON. 1970. Malignant catarrhal fever in Texas cervids. J. Wildl. Dis. 6: 367-383.
4. ——, and L.G. ADAMS. 1976. Viral particles associated with malignant catarrhal fever in deer. Am. J. Vet. Res. 37: 837-840.
5. HAMDY, F.M., A.H. DARDIRI, C. MEBUE, R.E. PIERSON and D. JOHNSON. 1978. Etiology of malignant catarrhal fever outbreak in Minnesota. Presented at the 82nd Ann. Meet. U.S. Anim. Health Assoc., Buffalo, New York.

6. HATKINS, J. 1980. Endemic malignant catarrhal fever at the San Diego Wild Animal Park. J. Wildl. Dis. 16: 439-443.
7. HUCK, R.A., A. SHAND, P.J. ALLSOP and A.B. PATERSON. 1961. Malignant catarrhal fever of deer. Vet. Rec. 73: 457-465.
8. PIERSON, R.E., J. STORZ, A.E. MCCHESNEY and D. THAKE. 1974. Experimental transmission of malignant catarrhal fever. Am. J. Vet. Res. 35: 523-525.
9. PLOWRIGHT, W., F.R. MACADAM and J.A. ARMSTRONG. 1964. Growth and characterization of the virus of bovine malignant catarrhal fever in East Africa. J. Gen. Microbiol. 39: 253-266.
10. ———. 1968. Malignant catarrhal fever. J. Am. vet. med. Ass. 152: 795-806.
11. RWEYEMAMU, M.M., L. KARSTAD, E.Z. MUSHI, J.C. OTEMA, D.M. JESSETT, L. ROWE, S. DREVEMO and J.G. GROOTENHUIS. 1977. Malignant catarrhal fever virus in nasal secretions of wildebeest: A probable mechanism for virus transmission. J. Wildl. Dis. 10: 478-487.
12. SANFORD, S.E. and P.B. LITTLE. 1977. The gross and histopathologic lesions of malignant catarrhal fever in three captive sika deer *(Cervus nippon)* in Southern Ontario. J. Wildl. Dis. 13: 29-32.
13. WYAND, D.S., C.F. HELMBOLDE and S.W. NIELSON. 1971. Malignant catarrhal fever in white-tailed deer. J. Am. vet. med. Ass. 159: 603-610.
14. ZIMMER, M.A., C. PATRICK MCCOY and J.M. JENSEN. 1981. The African form of malignant catarrhal fever in captive Indian gaur: Comparative gross and histopathologic studies of MCF in Indian gaur and domestic cattle. J. Vet. Path. (Submitted).

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