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Author: HOFF, GERALD L.

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EXPERIMENTAL INFECTION IN NORTH AMERICAN ELK WITH EPIZOOTIC HEMORRHAGIC DISEASE VIRUS[1]

GERALD L. HOFF, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin, 53706, U.S.A.[2]

DANIEL O. TRAINER, Dean, College of Natural Resources, University of Wisconsin, Stevens Point, Wisconsin, 54481, U.S.A.

Abstract: Two elk *(Cervus canadensis)* were inoculated intravenously with epizootic hemorrhagic disease virus. No overt signs of disease were observed in the elk although one animal had a slight febrile response. A cell associated viremia was detected in both elk, which persisted in one animal for 30 days post-inoculation. The similarities of these results to those reported for bluetongue virus infection in elk is discussed.

INTRODUCTION

The natural host range of the virus of epizootic hemorrhagic disease of deer (EHDV) has not been completely defined. Fatal epizootics of EHDV have been reported in white-tailed deer *(Odocoileus virginianus)*, mule deer *(O. hemionus)* and pronghorn antelope *(Antilocapra americana)*.[13] However, attempted experimental infections in one moose *(Alces alces)*[3] and in domestic sheep *(Ovis aries)*[1] have failed to produce disease.

This study was undertaken to determine the susceptibility and response of North American elk *(Cervus canadensis)* to experimental infection with EHDV.

MATERIALS AND METHODS

Two elk were used in this study. E-1 was an adult female which had originally been obtained from a commercial "game farm" in Wisconsin and was maintained at the University of Wisconsin Charmany Research Center for 2 years prior to this experiment. The second elk, E-2, was a 10 month-old female born to E-1 and reared at the Charmany Center.

Two weeks prior to the initiation of the experiment, the elk were moved into separate isolation facilities where they were kept for the duration of the experiment.[9] Pre-exposure serum samples were collected when the elk were moved and also just prior to inoculation. Additional serum samples were collected on post-inoculation days (PID) 7 to 14, 30 and 170. The sera were heat-inactivated at 56 C for 30 minutes and tested for the presence of neutralizing antibodies to EHDV and bluetongue virus (BTV) using the plaque reduction neutralization test.[14,16]

Both elk were inoculated intravenously with 3 ml of white-tailed deer blood containing 10^3 tissue culture infective doses ($TCID_{50}$) of EHDV per ml. The virus was originally isolated from a North Dakota white-tailed deer which died during an EHDV epizootic in the fall of 1970 (manuscript in preparation).

The elk were observed daily for signs of disease, overt or clinical. Blood samples were collected and rectal temperatures recorded daily for the first 14 days and then every other day until PID 30. An additional bleeding was made on

[1] Supported by funds from the Welder Wildlife Foundation, Sinton, Texas, 78387, U.S.A.

[2] Present address: Veterinary Public Health Section, Florida Division of Health, P.O. 210, Jacksonville, Florida, 32201, U.S.A.

[3] Karstad, L. H. 1971. Personal Communication. Ontario Veterinary College, Guelph, Ontario, Canada.

PID 170. Thirty ml of blood were collected from the jugular vein of each elk at every bleeding. The blood samples were combined 1:1 with an anticoagulant preservative solution of oxalate-phenol-glycerine (OPG).[4],[5]

An aliquot of each blood sample was diluted 1:10 in Hank's balanced salt solution (HBSS) and assayed for the presence of virus. The remainder of each blood sample was fractionated by centrifugation[6] within 1½ hours after each bleeding to yield a plasma-OPG fraction, a red-blood cell (RBC) fraction and a white-blood cell (WBC) fraction. The WBC fraction was then washed once in phosphate buffered saline (PBS) to remove any plasma, while the RBC fraction was washed three times in PBS. The cell fractions were diluted 1:10 in HBSS and treated with ultra sound by a Branson sonifer for 30 seconds with an attached ⅛ micro-tip horn at an instrument setting of 4.[7],[8] The plasma-OPG fraction was similarly treated but left undiluted.

The virus isolation attempts were made in monolayer cultures of BHK-21 cells utilizing the methodology of Thomas and Trainer.[10] Individual 0.1 ml portions of the blood samples were inoculated onto drained monolayer cultures and the cultures were observed for cytopathic effect (CPE). Samples which produced CPE were then titrated. Isolated agents were considered EHDV if they were neutralized by reference EHDV antiserum.

Serum samples were also obtained from 33 elk which were live-trapped in northern Idaho in the spring of 1969. The serum samples were heat-inactivated and were initially tested for EHDV neutralizing antibodies at 1:100 dilution. Negative samples were retested at 1:40. The original Shope isolate, NJ-55, of EHDV was used as the test virus at 30-80 plaque forming units per plate. Only sera which caused a reduction of 50% or greater in plaque count, when tested at either dilution, were considered positive.

RESULTS

Following experimental exposure to EHDV, no overt signs of disease were detected in the elk. E-2 did have a slight rise in temperature of approximately 1 C from PID 3 to 5.

Virus was recovered from the whole blood in OPG and from the blood fractions of both animals. If only the whole blood in OPG is considered, then the viremias lasted from PID 4 to 8 in E-1 and from PID 4 to 7 in E-2, with peak titers of $10^{4.0}$ $TCID_{50}$ and $10^{3.5}$ $TCID_{50}$/0.1 ml of inoculum respectively (Table 1). However, upon fractionation of the blood samples, the virus appeared to primarily be associated with the RBC fraction with viremia lasting from PID 4 to 30 in E-1 and from PID 3 to 16 in E-2. No virus was detected at PID 170 from either elk.

The pre-exposure serum samples from E-1 and E-2 were free of detectable neutralizing antibodies to both EHDV and BTV. Neutralizing antibodies to EHDV were first detected on PID 13 in E-2 and PID 18 in E-1. On PID 30, E-1 had a titer of 1:1280 which persisted to PID 170. E-2 on the other hand, had a decrease in titer from 1:640 on PID 30 to 1:80 on PID 170. None of the serum samples possessed neutralizing activity to BTV.

None of the Idaho elk sera reacted positively for EHDV neutralizing antibodies when tested at either 1:40 or 1:100.

DISCUSSION

Elk experimentally infected with EHDV respond in a manner similar to that reported for elk experimentally infected with BTV.[9] With either virus, overt disease was not produced and clinical disease, as measured by febrile response, was mild in young elk and inapparent in adults. The titers and durations of the resulting viremias (whole blood in OPG) were approximately the same in elk inoculated with BTV or EHDV, as was the

[4] OPG—Potassium oxalate, 5 gm; phenol, 5 gm; glycerol, 500 ml; and double distilled water, 500 ml.

TABLE 1. Distribution of EHDV in blood components of experimentally infected elk.

	Elk E-1				Elk E-2			
PID*	Whole blood in OPG	Plasma	WBC	RBC	Whole blood in OPG	Plasma	WBC	RBC
0	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
4	2.5**	—	2.7	3.0	1.7	—	trace	2.3
6	3.0	trace	2.5	3.8	3.8	1.3	2.7	5.0
8	2.8	—	2.5	3.5	—	—	—	3.3
10	—	—	1.8	3.0	—	—	2.3	2.5
12	—	—	—	2.7	—	—	—	2.0
14	—	—	—	2.0	—	—	—	2.3
16	—	—	—	1.7	—	—	—	2.3
18	—	—	—	2.5	—	—	—	—
20	—	—	—	1.0	—	—	—	—
22	—	—	—	2.3	—	—	—	—
24	—	—	—	2.5	—	—	—	—
26	—	—	—	2.6	—	—	—	—
28	—	—	—	1.7	—	—	—	—
30	—	—	—	2.0	—	—	—	—
170	—	—	—	—	—	—	—	—

* PID—post-inoculation day.

** Titers expressed as Log_{10} $TCID_{50}$ per 0.1 ml of inoculum. The inocula are serial dilutions of blood fraction and were not resuspended to the original blood volumes.

time of appearance of the respective virus-specific antibodies.

Of interest, is the association of EHDV with the cell components of the blood even in the presence of neutralizing antibodies. The EHDV-erythrocyte viremia is not unique as similar virus-RBC associations have been reported for BTV, Rift Valley fever, Colorado tick fever, Sendai, hog cholera, African swine fever and lymphocytic choriomeningitis. [3,4,5,6,9,11,12] However, for most of these viruses it is not known whether the viruses are situated intracellularly or are adsorbed to the surface of the erythrocytes. An intracellular location would permit the virus to escape the neutralizing effect of serum antibodies, as has been demonstrated with Sendai[3] and Colorado tick fever[9] viruses and postulated to occur with BTV.[4]

The persistence of EHDV-erythrocyte viremia up to PID 30 in E-1 is similar to observations made with BTV in sheep, goats, and cattle,[4] however, the loss of circulating virus in E-2, a few days after the appearance of EHDV-specific neutralizing antibodies, is more consistent with results obtained in white-tailed deer infected with either BTV or EHDV (manuscript in preparation).

Because neither EHDV epizootics nor sero-positive reactors have been reported in elk it is difficult to postulate what role elk may play in the epizootiology of the virus. The significance of the resulting viremia following experimental infection, in terms of the transmission of the virus, is speculative since, although EHDV is believed to be transmitted by hematophagous arthropods, there is little evidence to support this hypothesis. An EHDV-like virus has been isolated from *Culicoides* spp. in Nigeria, however the virus also cross-reacted by complement-fixation with BTV.[7] Additionally, inoculation of *Culicoides variipennis* with EHDV has resulted in infection in some of the gnats but transmission remains to be demonstrated.[5] Also, the question remains of whether or not the virus in its erythro-

[5] Luedke, A. J. 1970. Personal Communication. U.S. Dept. of Agriculture, Denver, Colorado, U.S.A.

cytic viremic stage, in the presence of EHDV neutralizing antibodies, is utilizable by an insect vector. Studies with *Aedes aegypti* mosquitces and yellow fever virus demonstrated that the mosquitoes were unable to become infected with virus when it was mixed with specific antiserum,[10] however a different vector-virus combination may yield different results.

At the present time, elk could be considered possible amplifying or short-term reservoir hosts for EHDV. Only through more comprehensive studies, especially serological can the status of elk in the epizootiology of EHDV be determined.

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