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# TRANSMISSION OF TWO STRAINS OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS IN DEER BY *Culicoides variipennis*

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*Abstract:* Two strains of epizootic hemorrhagic disease virus (EHDV), New Jersey (NJ) and Kentucky (KY), of deer were biologically transmitted between white-tailed deer, *Odocoileus virginianus,* by *Culicoides variipennis.* The KY strain, isolated from *C. variipennis* collected during an epizootic in deer, was identified as EHDV by serological tests. Deer exposed to the KY or the NJ strains of EHDV developed an acute hemorrhagic disease; most deer died 6 to 13 days after infection. Sheep inoculated with EHDV developed no clinical signs of disease.

## INTRODUCTION

*Culicoides variipennis* was indicated as the probable vector of a 1971 epizootic in penned white-tailed deer (*Odocoileus virginianus*) in Kentucky on the basis of epizootical data and virus isolations from parous females.[10] The epizootic was diagnosed as hemorrhagic disease[16,17] caused by epizootic hemorrhagic disease virus (EHDV) or bluetongue virus (BTV), both of which produce clinically and pathologically similar conditions in deer.[11,20] Thomas *et al.*[19] presented serological evidence that both EHDV and BTV were present during the 1971 epizootic among penned deer at Mammoth Cave, Kentucky.

BTV and EHDV are antigenically different although morphologically indistinguishable.[2,15,18] The similarities of these two viruses have led Borden *et al.*[2] and Murphy *et al.*[15] to include them in a new taxonomic group, the orbiviruses, with BTV as the prototype.

BTV has been biologically transmitted by *C. variipennis* between sheep[5] and cattle.[13] Boorman and Gibbs[1] recently showed that EHDV replicated to high titer in *C. variipennis,* with a growth cycle similar to that of BTV. Lee *et al.*[12] isolated BTV and EHDV from *Culicoides* spp. collected at Ibadan, Nigeria. Moore[14] detected group-reactive antigens between the Ibadan strains of EHDV and BTV by complement fixation and agar gel precipitin tests but not by neutralization tests.

We believed that *C. variipennis* could be a vector of EHDV. Therefore, experiments were conducted to determine if two of the EHDV isolates from Kentucky and the standard New Jersey strain of EHDV could be biologically transmitted between deer by *C. variipennis.*

## MATERIALS AND METHODS

### Virus

Parous female flies that had not had a recent blood meal were segregated from 1971 light trap collections[10] at Mammoth Cave, Kentucky for virus assay in embryonating chicken eggs.[6] Each pool of flies (133 flies in 22 pools) was ground in a TenBroeck grinder with 4 ml of PBS that contained 10,000 units of penicillin, 200 mg of streptomycin, and 1,000 units of mycostatin per ml. Each fly suspension was inoculated intravascularly into 20 ten-day eggs, 0.1 ml

per egg. Test incubation was at 33.6 C with daily candling for 7 days at which time all eggs with live embryos were discarded. Dead embryos from each sample were collected, pooled, blended, and then clarified by low-speed centrifugation. The resulting supernatant fluid was inoculated into the yolk sac of twenty 7-day eggs, 0.1 ml per egg. These test eggs were also incubated at 33.6 C but were candled daily through the 10th day at which time all live embryos were discarded. Embryos that died through the 10th day were processed as above. Two of these isolates, designated as KY 1 and KY 2, were inoculated into deer.

The NJ strain of EHDV, obtained as 2nd passage suckling mouse brain,[1] was also inoculated into deer for virus transmission tests.

#### Animals

Orphaned white-tailed deer were obtained as fawns from the southeastern U.S.[2] and maintained at our laboratory until their use at 8 to 10 months of age. Yearling Warhill wethers from a closed flock of known BTV-susceptible sheep also were used. Tests with virus-exposed animals were conducted in screened rooms under insect-proof conditions. Leukocyte counts and body temperatures were recorded daily until death of the animal or, if it survived, for at least 28 days after exposure to infection. Each deer, when used in a transmission trial, was tranquilized with 20-50 mg of xylazine.[3] [4]

#### Serology

The modified indirect fluorescent antibody (FAb) test[7] was used to identify the KY isolates and to confirm the identity of EHD NJ virus.

Serums of deer were tested for EHDV and BTV antibody by plaque neutralization (PN), complement fixation (CF), and agar gel precipitin (AGP) tests before and after infection. The preinfection (normal) serums were obtained before each deer was used in a test and the postinfection serums were obtained just before death or, if the deer survived, at 28 days postinfection.

The CF test was conducted according to the protocol of Veterinary Service Laboratories, APHIS, USDA, NADC, Ames, Iowa; this test is patterned after the technique for the modified direct CF test described by Boulanger.[3] AGP tests were conducted on the preinfection and postinfection serums after the method of Jochim and Chow.[8] PN tests[4] were conducted by mixing varying dilutions of serum with a constant amount of test virus.[5]

#### Insect Vector

Females from a colony of *C. variipennis,* Sonora strain 000-line,[9] were used in these transmission experiments as previously described.[5] Initial donor deer inoculated with EHD NJ and EHD KY viruses were bled during their viremic periods and flies were given a meal on the defibrinated blood through a membrane. These two groups of EHDV-exposed insects were extrinsically incubated at 22 C for 18 and 20 days at which time each group took a blood meal on a recipient host deer. These recipient hosts were in turn used as infecting donors by blood feeding normal flies directly on the animal followed by extrinsic incubation at 22 C for 14 days of the virus-exposed flies that were then fed on normal recipient host deer. EHDV-infected deer

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[1] American Type Culture Collection, Rockville, Maryland 20852, USA.

[2] Dr. F. A. Hayes, Southeastern Cooperative Wildlife Disease Studies, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA.

[3] Rompun: Chemagro, Division of Baychem Corporation, Kansas City, Missouri 64120, USA.

[4] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee of warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

[5] EHD Alberta from Dr. Lars Karstad, Ontario Veterinary College, Guelph, Ontario, Canada.

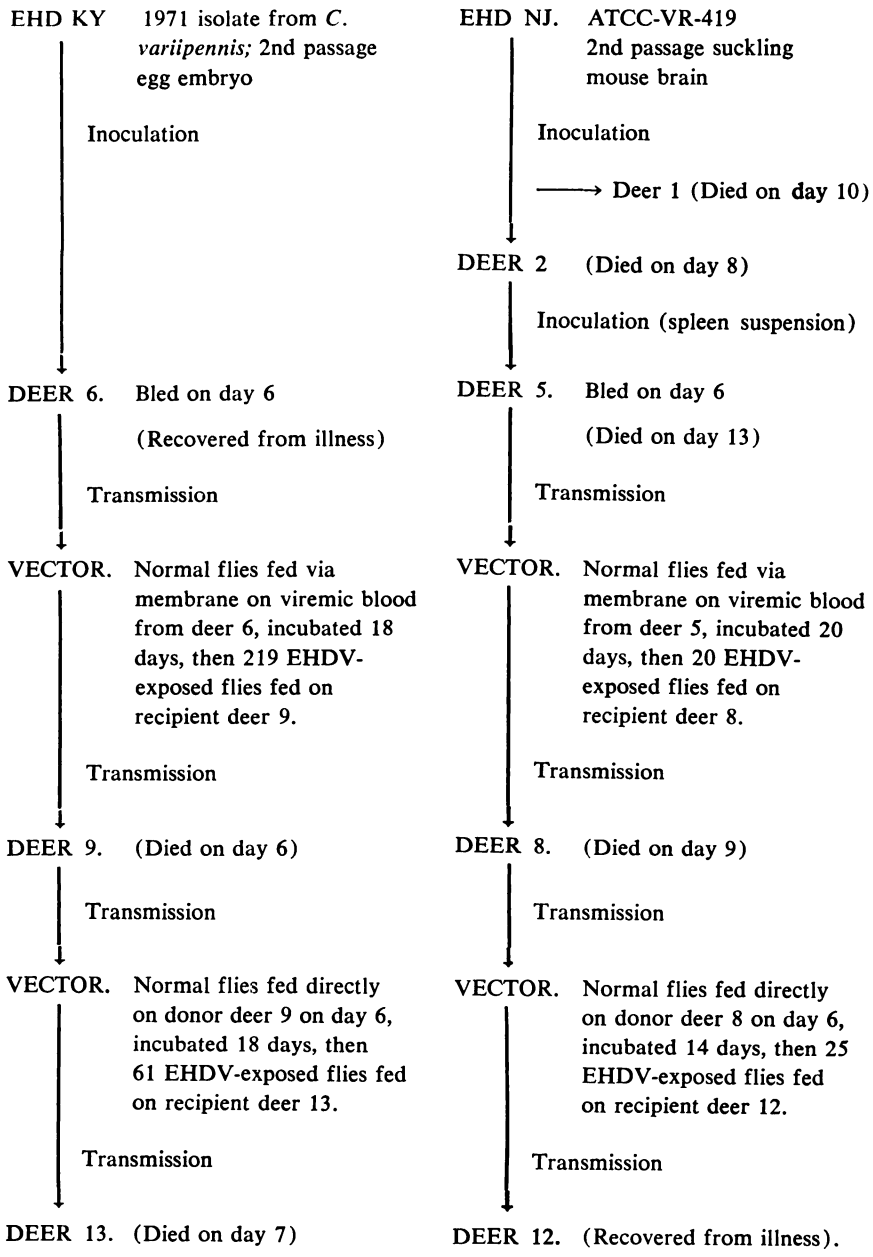


FIGURE 1. Serial transmission of epizootic hemorrhagic disease virus (EHDV) by **Culicoides variipennis** in deer.

were used as donors when the virus concentration was expected to be the greatest, i.e., at the time of peak febrile response and corresponding leukopenia that occurred 6 days after infection of the deer (Fig. 1).

## RESULTS

### Transmission

*C. variipennis* harbored EHDV after feeding on deer infected with either the NJ or KY 1 strains and, with 14-20 days of incubation, biologically transmitted the virus to susceptible deer (Table 1). Not only was an acute hemorrhagic disease elicited with the NJ and KY 1 EHD viruses, but serial transmission was also demonstrated as the recipient host in one test was successfully used as the infecting donor in the next series.

### Pathology

All deer infected with EHD NJ or EHD KY 1 viruses developed an acute hemorrhagic disease with marked depression and recumbency followed by death in 6 of 8 animals by the 6th to 13th day after infection. Fever occurred as early as day 2 and increased to a high of 40.7 C on day 6; body temperatures then decreased to subnormal levels just before death. By day 6, the deer commonly developed hyperemia of the oral and nasal mucosa and conjunctiva, fever of 1 to 4 C, anorexia, reduced water intake, and an onset of ptyalism. This was followed (ca. day 7-9) by a generalized weakness and the tongue became hyperemic and swollen. The muzzle eroded shortly before death. Deer 13 had a marked submandibular edema on days 6 and 7.

Prominent pathologic changes found during necropsy examination were hemorrhages of variable size and severity, depending on acuteness and duration of the disease. Hemorrhages were generalized in all organs and tissues. Areas prominently involved were the subcutaneous, lymphatic, circulatory, and gastrointestinal tissues. Petechial, ecchymotic, and suffusion hemorrhages were found in the subcutaneous areas, especially along the neck and thorax. Many of the lymph nodes of the head, neck, and thorax not only were enlarged and edematous but also were black with hemorrhage. The gastrointestinal tract was severely involved and the contents of the small intestines were generally black with blood. Extensive subepicardial and subendocardial petechial and ecchymotic hemorrhages 1 to 20 mm in diameter were frequently seen on the heart and aorta.

None of the 4 sheep inoculated with EHDV (2 each with the NJ and KY 1 strains) developed any overt clinical signs of disease.

### Serology

Fourteen provisional viruses were isolated from the 22 pools of 133 *Culicoides* flies inoculated into eggs. The KY 1 isolate inoculated into deer 6 caused clinical signs of hemorrhagic disease and subsequently produced antibodies to EHDV but not to BTV (Table 2). The KY 2 isolate, inoculated into deer 7 did not cause any signs of disease although the postinfection serum revealed EHDV antibody (Table 2). The CF test, but not the PN test with the virus strains used, demonstrated BTV antibodies in the convalescent serum of deer 7. CF and PN tests, in contrast to the AGP test (Table 2), detected no antibody in any of the normal (preinfection) deer serums against EHD or BT antigen. CF, AGP, and PN tests showed little or no cross reactivity between EHDV antiserum and BT antigen (Table 2) except for the serum of deer 7 in the CF test. All infected deer that survived long enough (13 days) after infection produced EHDV antibody detectable by each of the 3 serologic tests. BTV and EHDV were differentiated by the indirect FAb test[7] and KY isolates 1 and 2 were identified as EHDV (Table 3).

## DISCUSSION

EHDV, a member of the orbivirus genus for which BTV is the type species, was biologically transmitted by the insect vector *C. variipennis*. Because *Culicoides* spp. has been shown to be the

TABLE 1. Serial epizootic hemorrhagic disease virus (EHDV) transmission tests (pools of **Culicoides variipennis** were fed on infected donor deer, incubated at 22 C, and refed on recipient host deer).

| | Donor deer | | | | Recipient host deer | | |
|---|---|---|---|---|---|---|---|
| Virus | Deer No. | Method of infection | Day flies fed after infection | No. of days EHDV-exposed flies incubated | Deer No. | No. of exposed flies fed | Transmission results |
| EHD NJ | 5 | Inoculation | 6* | 20 | 8 | 20 | Positive |
| | 8 | Bites of 20 exposed flies | 6 | 14 | 12 | 25 | Positive |
| EHD Ky 1 | 6 | Inoculation | 6* | 18 | 9 | 219 | Positive |
| | 9 | Bites of 219 exposed flies | 6 | 14 | 13 | 61 | Positive |

*Flies were fed through membranes on infected blood withdrawn from deer 5 and 6.

TABLE 2. Complement fixation, agar gel precipitin, and serum neutralization results on sera collected before and after infection on donor and recipient deer.

| Serum | | Antigen | | | | | |
|---|---|---|---|---|---|---|---|
| | | Complement Fixation | | Agar Gel Precipitin | | Plaque Neutralization | |
| Deer Number | Day of Infection | EHD NJ | BT 8 Ca | EHD NJ | BT 8 Ca | EHD Alberta | BT 8 Ca |
| EHD Ky 1 | | | | | | | |
| 6 | Pretest | <5 | <5 | Negative | Negative | <8 | 8 |
| 6 | 28 | 80 | <5 | Positive | Negative | 32 | 8 |
| 9 | Pretest | <5 | <5 | Suspicious | Negative | <8 | 8 |
| 9 | 6 (Died) | <5 | <5 | Positive | Negative | <8 | 16 |
| 13 | Pretest | | | | | | |
| 13 | 7 (Died) | No sera collected | | | | | |
| EHD Ky 2 | | | | | | | |
| 7 | Pretest | <5 | <5 | Negative | Positive | <8 | ≦8 |
| 7 | 28 | ≧160 | 40 | Positive | Positive | 2048 | 16 |
| EHD NJ | | | | | | | |
| 5 | Pretest | <5 | <5 | Negative | Negative | <8 | 8 |
| 5 | 13 (Died) | 40 | 5 | Suspicious | Negative | 2048 | 8 |
| 8 | Pretest | <5 | <5 | Suspicious | Negative | <8 | ≦8 |
| 8 | 9 (Died) | <5 | <5 | Suspicious | Negative | 128 | 16 |
| 12 | Pretest | <5 | <5 | Positive | Negative | <8 | ≦8 |
| 12 | 28 | 40 | <5 | Positive | Negative | 512 | ≦8 |

TABLE 3. Indirect Fluorescent Antibody Test Results.

| Cell culture viral antigen | Rabbit Serum antibody | |
|---|---|---|
| | EHD NJ | Bluetongue |
| EHD NJ | Positive | Negative |
| EHD KY 1 | Positive | Negative |
| EHD KY 2 | Positive | Negative |
| BT 8 Ca | Negative | Positive |

principal, if not the only vector for BTV, it probably is also the primary vector for EHDV. The principal method for differentiating EHDV from BTV resides in their serologic (antigenic) distinctness and differences in their host range. In addition to separation of EHDV and BTV by CF, AGP, PN and indirect FAb tests, sheep, which are susceptible to BTV but are overtly refractive to EHDV, could serve as a differential host for the two viruses.

Deer 6, 7, and 12, in contrast to the other 5 deer, survived infection with EHDV. Deer 6, although it suffered an acute illness of EHD, was inoculated with virus that could have become less virulent as a result of subpassage from insects into eggs. Although the pretest (normal) serums from deer 7 and 12 were BT and EHD negative by the CF and PN tests, the AGP test was positive for BT (Deer 7) and EHD (Deer 12) which suggest a passive experience with these agents before acquisition of the animals. Deer 7, unlike all the other deer that had an acute response to the virus, had an inapparent reaction supported by positive CF, PN, and AGP tests on the postinfection serum. Because no BTV antigen was indicated by the indirect FAb test in the sample inoculated into deer 7, the increase in BTV antibody titer via CF test may have been the result of an anamnestic response to a similar antigen.

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