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IDENTIFICATION OF VIRUSES INVOLVED IN THE 1971 OUTBREAK OF HEMORRHAGIC DISEASE IN SOUTHEASTERN UNITED STATES WHITE-TAILED DEER

F. C. THOMAS, N. WILLIS and G. RUCKERBAUER[1]

Abstract: Tissues and serum from white-tailed deer (*Odocoileus virginianus*) in the southeastern United States were taken during an outbreak of hemorrhagic disease. These were subjected to virus isolation attempts in tissue culture and several serological tests (plaque neutralization, complement fixation, agar gel precipitation). Both epizootic hemorrhagic disease of deer virus (EHDV) and bluetongue virus (BTV) were isolated and evidence of their presence demonstrated serologically. All of the evidence supported previous suggestions that these two agents are antigenically distinguishable.

INTRODUCTION

During the summer of 1971, a widespread disease of white-tailed deer was observed in the southeastern United States.[2] Samples (tissue and serums) were supplied to the Animal Diseases Research Institute[1] by the Southeastern Cooperative Wildlife Disease Study[2] (SCWDS) and the U.S. Bureau of Sport Fisheries and Wildlife[3] (BSFW). These included samples from wild deer (SCWDS) and both native and newly introduced penned deer (BSFW). The latter were sentinel deer which were serologically negative for bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). The clinical, pathological and epidemiological observations will be reported by other investigators.[2,4]

MATERIAL AND METHODS

Five sets of blood samples and other tissues (from deer no. 1 to 5) of which three (No. 1, 2, 3) were accompanied by serum, were supplied by SCWDS. All five deer were dead or affected by the described syndrome.[2,4] Blood samples (for virus isolation) were preserved with equal volumes of OPG.[5] Serum and other tissues were shipped frozen.

Serum samples were received from BSFW and included 14 from penned deer (all normal or convalescing) in the Mammoth Cave, Kentucky area.

Blood and pooled tissue suspensions (usually spleen, liver, heart, lung and kidney) submitted for virus isolation were inoculated onto L-cells[3] and WI2[3] cells. The isolates were identified by neutralization,[5] plaque type,[6] and fluorescent antibody staining.[1] In one case (tissue pool 3) two viruses were separated by the conditions of their replication in W12 cells, without the use of antiserum. They were identified by a fluorescent focus technique employing a gum tragacanth overlay[8] and specific conjugates.[1] Serum samples were tested with one or more of

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[3] U.S. Bureau of Sport Fisheries and Wildlife, Mammoth Cave, Kentucky, and Denver Wildlife Research Center, Denver, Colorado.

[4] Roughton, R. 1974. An outbreak of a hemorrhagic disease of white-tailed deer in Kentucky. Manuscript in preparation.

[5] O.P.G. — potassium oxalate, 5 ml; phenol, 5 gm; glycerol, 500 ml; Distilled water, 500 ml.

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the following tests for EHDV and BTV antibodies; plaque neutralization[4] (PN), complement fixation[1] (CF) and agar gel precipitation[1] (AGP).

RESULTS

SCWDS Samples:

BTV was isolated from blood 2 and EHDV from blood 3, the others being negative. Tissue pool 1 yielded no virus, pools 2 and 4 BTV, pool 3 both BTV and EHDV and pool 5 EHDV. These isolations were repeatable.

Serum 1 was EHDV antibody positive, serum 2 BTV antibody positive and serum 3 negative (Table 1).

BSFW Samples:

Of nine native deer serums from this group, one was positive for BTV and EHDV, two questionable for BTV and positive for EHDV and six negative for both viruses. The five samples from the sentinel (introduced) deer yielded one questionable for EHDV, three questionable for BTV and positive for EHDV and one positive for EHDV (Table 2).

TABLE 1. Virus isolation and serological results from samples supplied by Southeastern Cooperative Wildlife Disease Study.

Specimen	Virus Isolation (blood)		Virus Isolation (tissues)		Serology	
	BTV	EHDV	BTV	EHDV	BTV	EHDV
1	—	—	—	—	—[a]	+(CF,AGP,PN)
2	+	—	+	—	+(AGP,PN)	—
3	—	+	+	+	—	—
4	—	—	+	—	ND[b]	ND
5	—	—	—	+	ND	ND

[a] negative in all 3 tests (CF, AGP, PN).
[b] ND — not done

TABLE 2. Serological results on samples supplied by the U.S. Bureau of Sport Fisheries and Wildlife.

	Serology	
Resident Penned Deer	BTV	EHDV
MC-109	+(PN)	+(CF, AGP, PN)
MC-125	Q[a](PN)	+(PN)
MC-141	Q(PN)	+(CF, AGP, PN)
Remainder (6 samples)	—[b]	—
Sentinel Deer		
MC-236	—	Q(PN)
MC-237	Q(PN)	+(PN)
MC-238	—	+(CF, AGP, PN)
MC-239	Q(PN)	+(CF, AGP, PN)
MC-240	Q(PN)	+(CF, AGP, PN)

[a] questionable.
[b] negative in all 3 tests (CF, AGP, PN).

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These results and an indication of the tests on which our conclusions were based are given in Tables 1 and 2.

DISCUSSION

The data indicate that both BTV and EHDV were active during the 1971 deer die-off. Inasmuch as both viruses have been experimentally shown to be capable of producing hemorrhagic lesions and mortality in white-tailed deer,[3,7] the laboratory findings suggest these to be the most likely candidates.

It is interesting that both virus isolations and serology confirmed previous suggestions that the viruses are antigenically distinguishable.[5,6] One could hypothesize that climatic conditions (i.e. those favouring biting anthropods) allowed BTV and EHDV already present in the area to infect large numbers of animals, some of which died.

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