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Experimental Exposure of Deer to California Encephalitis Virus

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Abstract

To determine the susceptibility of white-tailed deer *(Odocoileus virginianus)* to California encephalitis virus (CEV), four captive deer were experimentally exposed by different routes and the clinical, virological and serological responses recorded. None of the four deer developed a detectable viremia or clinical signs of disease. One deer exposed subcutaneously and one exposed intravenously developed circulating antibody which persisted; the deer exposed intranasally and orally did not. Rechallenge of a deer with detectable antibody resulted in a typical anamnestic antibody response. The significance of CEV to deer and the possibility of utilizing deer as sentinels for CEV monitoring are discussed.

The most prevalent viral antibody detected in the white-tailed deer population on the Welder Wildlife Refuge, Sinton, Texas, was that of the California encephalitis virus group (CEV). Over a 3 year period (1963-66) approximately 46 percent of the 249 adult deer tested were positive[1]. Trainer (personal communication) found a similar high prevalence of CEV antibody in Wisconsin deer.

Hammon and Sather[2] and Sather and Hammon[5] have recently summarized the history of the CEV complex and they concluded that there are at least 11 types of virus in the CEV group in the United States and Canada. These differ in prevalence from year to year as well as from season to season, presumably the result of different vectors and host ranges.

Since the host and geographic range of the CEV group is quite broad[2, 5, 7] and since it appears to be prevalent in deer of Texas and Wisconsin, it seemed desirable to determine the susceptibility of deer to the virus. White-tailed deer were experimentally exposed to CEV by several routes and the clinical, virological, and serological responses recorded.

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Materials and Methods

Four serologically negative (CEV) white-tailed deer, 2 females approximately 5 months of age and 2 males about 6 months of age, housed in isolation units at the Charmany Farm Research Center were exposed to CEV.

One female (No. 1) was inoculated subcutaneously (SC) in the right hip; the other female (No. 2) received the inoculum intravenously (IV) in a jugular vein; one male (No. 3) was exposed intranasally, the inoculum being dropped slowly into the nostrils; and the other male (No. 4) received the inoculum orally in a gelatin capsule. Each deer received 3 ml of inoculum (10^7 MLD_{50}/ml) which was a 10-percent suckling-mouse brain suspension diluted with 5-percent bovine-plasma albumin in sterile, buffered, double-distilled water plus 1,000 units of penicillin and 1,000 mg of streptomycin per ml. The CEV used was the same isolate used in the metabolic inhibition test described below, the Montana snowshoe hare isolate, 1959, in its 18th to 19th mouse-passage level.

Body temperatures were recorded daily beginning 1 week prior to challenge and ending 10 days post-inoculation (PI). The animals were examined daily for clinical signs of disease.

Each deer was bled from a jugular vein prior to exposure, then daily for 10 days PI, and then periodically. Virus-isolation attempts were made daily during the 10-day PI period, using the following method: immediately after the blood was drawn, three ten-fold dilutions were made in tryptone broth, and undiluted blood as well as each of the three dilutions was inoculated IC (0.02 ml) into five 3-day-old suckling mice. The mice were observed daily for 14 days for central nervous system disorders or death. Serum was separated from the remainder of the blood sample and was used for serologic testing.

The metabolic-inhibition (MI) test was utilized to screen all experimental serums. This was the same test used to examine serums of the wild deer populations. This neutralization test utilized HeLa-cell cultures in multiple-cup plastic panels in a manner devised by Melnick and Opton [4] and later adapted for arboviruses by Kuns[3]. Selected serums were also titrated in the MI test.

In addition, selected deer serums were also tested at the Wisconsin Laboratory of Hygiene with mouse neutralization (MN), hemagglutination inhibition (HI) and complement fixation (CF) tests following the procedures of Thompson and Evans[6] and the results compared with those of the MI test. In the MN tests, serums that neutralized 1.7 logs LD_{50} or more were considered positive. In the HI test serums with antibody titers of 1:10 or higher were considered positive. All CF tests attempted produced anticomplementary reactions.

Deer No. 2, which originally received the IV inoculation, was rechallenged SC with a similar CEV suspension 16 months after initial exposure to observe if a secondary (anamnestic) antibody response would develop. Temperatures were again recorded 1 week prior to inoculation and subsequently for 10 days. Blood samples for serology were taken daily for 10 days PI and periodically thereafter.

Results

Clinical signs of disease were not observed in any of the four experimental deer at any time. Deer No. 1 died 7 months after exposure; however, the cause of death was not attributable to CEV.

All virus isolation attempts were negative.

MI test results of serums from the 4 deer showed that deer Nos. 1 (SC) and 2 (IV) developed detectable neutralizing antibody by days 5 and 4 PI respectively. Neutralizing antibody was not detected in any of the serums from deer Nos. 3 (IN) or 4 (oral).

The neutralizing antibody titer in deer No. 2 increased from the day of first appearance (day 4) to levels of 1:16 (serum dilution) on day 7 PI and continued at that approximate level for 16 months (Table 1). The deer was then rechallenged with CEV. The MI titer began to rise by day 4, and by 6 days PI it had reached 1:256, resembling a typical secondary antibody response.

The HI antibody titer of deer No. 2 was 1:10, considered positive in this test, on day 9 PI. Then the HI titer was below detectable levels until 6 days after rechallenge when the titer rose to 1:20 and remained at that level for at least 16 days.

The primary antibody response of deer No. 1 as measured in the MI test was similar to that of deer No. 2. No HI titer above 1:10 was detected in this animal.

TABLE 1. *Antibody response of deer No. 2 experimentally exposed intravenously with California encephalitis virus (Montana snowshoe hare isolate).*

Time after exposure	Test and antibody titer*		
	MI screening	MI titration	HI**
First exposure			
0 days	neg.	NT	neg.
4 days	+	NT	neg.
5 days	+	2	neg.
7 days	+	16	neg.
9 days	+	NT	10
10 days	+	NT	neg.
5 months	+	8	neg.
7 months	+	16	neg.
11 months	+	16	neg.
16 months	+	8	neg.
Second exposure			
2 days	+	4	neg.
4 days	+	16	neg.
6 days	+	256	20
8 days	+	256	20
21 days	+	NT	20

* Reciprocal of antibody titer; NT = no test run

**a 1:10 or higher serum dilution was considered positive

To further clarify the interpretation of CEV reactors in the MI test, selected CEV-positive deer serums from the field study were titrated in the MI, the MN, and the HI tests and the results compared (Table 2). Four serums and a negative control were tested in all four procedures, and the serum with the highest titer in the MI test had a correspondingly high titer in the MN test. Only the two highest-titered serums in the MN test evoked a 1:10 titer in the HI test. The control serum was negative in all tests.

Serums tested in both MI and HI tests were negative for WEV, EEV, SLE, and Powassan antibodies.

TABLE 2. *Comparison of California encephalitis virus antibody titers in deer serums titrated with three serologic tests.*

MI		MN	HI
(Mont SSH)*	(Mont SSH) Titration	(LaCrosse) Log neut**	(Cal BFS-283) ***
+	1:32	2.9	1:10
+	—	2.1	1:10
+	1:4	1.5	negative
+	1:2	1.3	negative
+	1:2	1.1	negative
neg. (control)	neg.	0.3	negative

* ( ) = California encephalitis virus strain used as antigen.
** 1.7 or higher logs of virus neutralized was considered positive, 1.0 - 1.6 logs considered presumptive, less than 1.0 logs negative.
***A 1:10 or higher serum dilution was considered positive.

Discussion

It is appreciated that care must be taken in interpreting serologic results that are not supported by virus isolations; however, the objective of the serologic study on the Welder Wildlife Refuge was to provide leads on the presence and prevalence of specific disease agents. The MI test was used in this study because large numbers of serums could be screened in a uniform, rapid, and inexpensive manner, and only small amounts of serum were needed. The sensitivity of the MI test for detecting CEV neutralizing antibody in humans has been established by Thompson and Evans[6].

In this laboratory study, comparison of serologic results of the MI, HI, and MN tests, showed a correlation and suggested that antibody detected with the MI screening test was produced by a California encephalitis group virus. The use of different strains of CEV as antigen in each of the three tests was recognized but was considered of minimal serologic importance for this study. Sather and Hammon[5] concluded that the California and Montana snowshoe hare strains cross-react with all strains sufficiently to be used as diagnostic antigens in conventional MN and CF tests.

A typical primary and secondary antibody response in white-tailed deer to CEV challenge provided further evidence of the specificity and sensitivity of the tests used (Table 1).

The lack of clinical signs and a detectable viremia suggests that the CEV inoculum used in this study produces an inapparent disease in white-tailed deer. Adaptation to a laboratory host, such as the mouse, has in many instances altered the ability of viruses to induce overt disease in other species. Also, experimental inoculation, even IV, does not precisely simulate arthropod transmission. As a result, failure to induce clinical disease must not be construed as evidence that natural exposure to unadapted virus of all members of the California complex will fail to induce clinical disease. The importance of CEV infections to white-tailed deer populations remains for the most part unknown.

California encephalitis has not been considered detrimental to wild populations; however, this idea may change as more information is compiled on the virus and its relationship to animal populations. Recent findings of Thompson *et al.*[7] have shown one strain of CEV to be capable of causing death in young children. Previously CEV was considered unimportant to human health.

The geographic as well as the host range of the CEV complex has expanded rapidly in recent years due to the accumulation of new information[2]. It is now known to be present over much of North America as well as in Europe and in a variety of wild and domestic species including man. Additional studies will undoubtedly expand these ranges even more.

Deer are the most important big game species in North America and they are present in every state. Since deer appear to be immunologically sensitive to CEV, they may act as good indicators of viral activity. Annual collections of serums from known-aged deer taken during hunting seasons could provide a useful index for monitoring the activity of this virus.

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ABSTRACT

JOHNSON, R. H. and R. E. W. HALLIWELL. 1968. Natural susceptibility to feline panleucopenia of the coati-mundi. Vet. Rec. *82:* 582.

The virus of panleucopenia was isolated in kitten kidney cell cultures from two captive coatis *(Nasua nasua).* The host range of panleucopenia virus strains is now known to include domestic cats, panthers, lions, leopards and tigers in the *Felidae,* mink in the Mustelidae, and coati-mundi in the *Procyonidae.* There is circumstantial evidence that the disease occurs also in raccoons.