ANTIBODY RESPONSES OF COYOTES INOCULATED WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS

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ANTIBODY RESPONSES OF COYOTES INOCULATED WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS

The geographical distribution of Venezuelan equine encephalitis (VEE) virus includes many South and Central American countries and the southeastern and possibly the western desert areas of the United States (Sidwell, *et. al.*, 1967. Bacteriol. Rev., 31:65-81). As summarized by these workers, the VEE virus has a wide host range that includes domestic animals, birds, a marsupial, bats, rodents, and carnivores. To this list has been added the coyote (Lundgren and Smart, 1968. Am. J. Trop. Med. and Hyg., in press) which has various subspecies distributed throughout most of North and Central America (Hall and Kelson, 1959. *Mammals of North America.* Vol. II:843-846. The Ronald Press Co.) where they range into areas where VEE is known to occur.

We reported that coyote pups, *Canis latrans lestes* Merriam, were susceptible to experimental infections with as few as $10^{1.7}$ 21-day old mouse intracerebral 50% lethal ($MICLD_{50}$) doses of VEE virus as determined by the development of a viremia (Lundgren and Smart, *op. cit.*). Because of the magnitude and duration of the viremia in the experimentally infected coyotes, it was concluded that this animal could have a role in the cycle of VEE in nature. To better understand the possible role of this animal in the epidemiology of VEE it was suggested that coyotes be included in future serological surveys for the incidence of VEE in wildlife, especially in areas where the virus is known to be endemic. To aid in interpreting the results of such a survey, the present study of the antibody responses of the experimentally infected coyotes mentioned above was undertaken using VEE and related antigens.

Materials and Methods

Animal collection and handling have been described (Lundgren and Smart, *op. cit.*). Briefly, the coyotes were divided into two different age groups which were in turn divided into subgroups of six animals each that were then inoculated with different ten-fold serial dilutions of VEE virus by the subcutaneous route. Fifty-four animals in one group (nine subgroups of six each) were inoculated when 1 to 2 months of age, and 18 pups in a second group (three subgroups of six each) were inoculated when 6 to 7 months of age. The younger coyotes were inoculated with virus concentrations ranging from $10^{1.7}$ to $10^{9.7}$ mouse intracerebral $MICLD_{50}$ and the older coyotes with $10^{0.2}$, $10^{3.2}$ and $10^{6.2}$ $MICLD_{50}$. Sera were collected from blood drawn from the jugular vein. All coyotes were bled prior to inoculation and at 1, 2, 3, 4 and 6 weeks after inoculation. Seven of the 1 to 2 month old coyotes were selected at random from the surviving animals and were also bled for sera at 7, 21, 31 and 41 weeks. The latter group consisted of one animal inoculated with $10^{9.7}$, three with $10^{8.7}$, two with $10^{5.7}$, and one with $10^{1.7}$ $MICLD_{50}$ doses of VEE virus. All sera were stored in a mechanical freezer at —65 C until tested. In most instances all of the different sera collected from one coyote were tested at the same time to minimize the minor day to day variations in serology.

The sera were tested for hemagglutination inhibiting (HI) with VEE, Western equine encephalitis (WEE) and Eastern equine encephalitis (EEE) antigens and VEE virus neutralizing (SN) antibody by standard procedures (Hammon and Work, 1964. In Lennette and Schmidt (ed.) *Diagnostic Procedures for Viral and Rickettsial Diseases*: 268-311. Third Ed. American Public Health Association, Inc.). The kaolin

adsorption technique was used for the treatment of sera for the HI test. The complement fixation (CF) test for VEE antibody was done according to the procedures outlined by Thorpe, *et al.*, 1965 (Proc. Soc. Exptl. Biol. and Med., 118:179-181).

Results and Discussion

The antibody response of the seven coyotes held for 41 weeks after the inoculation of VEE virus are illustrated in Figures 1 and 2. The VEE HI antibody responses were rapid with peak titers reached by one week after inoculation (Figure 1). The VEE CF antibody was not detectable until the second week and peak titers were reached by the third week. Significant VEE SN antibody levels were detectable by one week and reached peak neutralizing indices at three weeks. All antibody levels remained within the same general ranges during the 41 weeks that these animals were observed. These findings are similar to the antibody responses in man except that in humans the CF titer tends to decrease a few months after infection (Hammer and Work, *op. cit.* and Work, 1964. In Lennette and Schmidt (ed.) Diagnostic Procedures for Viral and Rickettsial Diseases: 312-355. Third Ed. American Public Health Association, Inc.).

In the coyotes antibody that cross reacted with WEE and EEE antigens in the HI test reached peak titers at three weeks as compared to the one week for the VEE antibody (Figure 2). The WEE and EEE HI titers were lower at the seventh week but the titers at 21 and 31 weeks remained in the same general range. At 41 weeks the WEE titers had decreased only slightly while nearly all of the EEE titers were negative. The magnitude of these cross reactions is similar to that observed in mice (Casals and Brown, 1954. J. Exptl. Med., 99:429-449). The VEE CF and HI antibody responses and the WEE and EEE HI cross reacting responses of all other coyotes, both the younger and older animals, that developed a viremia fell within the ranges of titers illustrated in Figures 1 and 2. No SN antibody determinations were made in these animals.

Two of the 1 to 2 month old coyotes that were in the subgroup that received the lowest dose of virus, $10^{1.7}$ $MICLD_{50}$, were the only animals in this group that did not develop a viremia (Lundgren and Smart, *op. cit.*). One of these coyotes developed only transient low levels of CF and HI titers and the second developed titers in the same ranges as those of the infected animals. Two of the 6 to 7 month old coyotes inoculated with the lowest dose of virus, $10^{0.2}$ $MICLD_{50}$, given in their age group did not develop a viremia. Both animals, however, developed CF and HI antibody titers at levels similar to the animals that had demonstrable viremia.

It is evident from this study that VEE virus infection in coyotes can readily be diagnosed by any one of the three methods used within at least 41 weeks of the initial exposure. These findings again support the need expressed by others for the use of more than one antigen when conducting a serological survey for the incidence of arboviruses (Hammon and Work, *op. cit.* and Sidwell, *et al., op. cit.*).

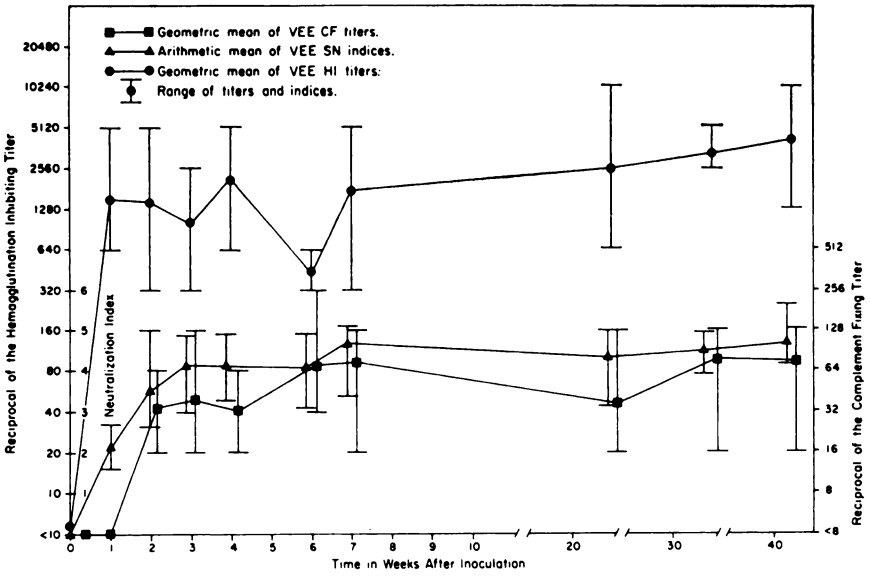

Figure 1 Complement fixing (CF), hemagglutination inhibiting (HI), and neutralizing (SN) antibody responses of seven coyote pups after the inoculation of Venezuelan equine encephalitis (VEE) virus

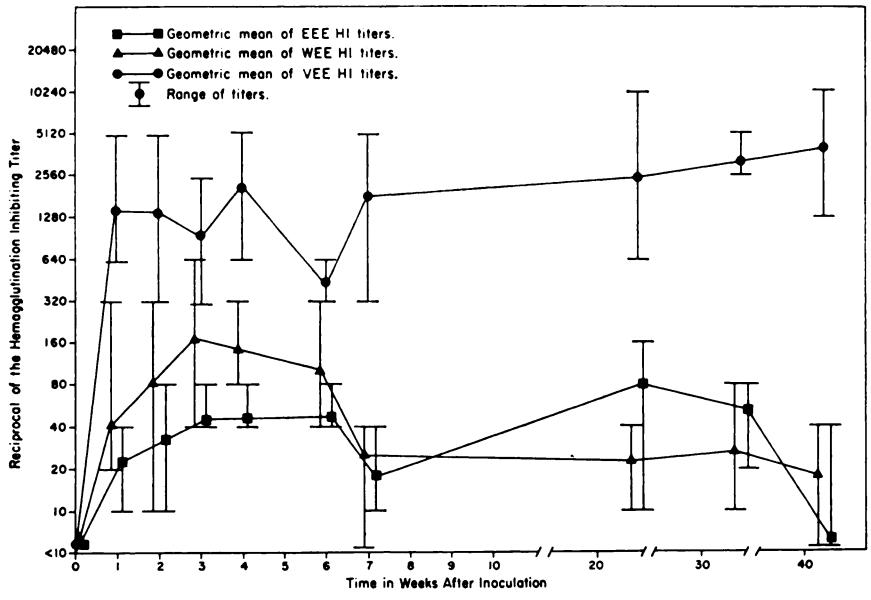

Figure 2. Hemagglutination inhibition (HI) of Venezuelan equine encephalitis (VEE), Western equine encephalitis (WEE) and Eastern equine encephalitis (EEE) viruses by the sera of seven coyotes inoculated with VEE virus.

Acknowledgements

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