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## VENEZUELAN EQUINE ENCEPHALOMYELITIS IN PHEASANTS AND CHUKARS

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**Abstract:** Pheasants (*Phasianus colchicus*) and chukars (*Alectoris graeca*) were inoculated with Venezuelan equine encephalitis virus. Antibody titers reached a peak 2 weeks postinoculation and then declined. Viremia was of short duration, clinical signs were not detected, and uninoculated cage mates did not develop antibody titers.

### INTRODUCTION

Birds are an important reservoir in the maintenance of many arthropod-borne encephalitis producing togaviruses. For example, pheasants, chukars, and ducks are all susceptible to eastern equine encephalitis (EEE) virus. Bird-to-bird transmission has been demonstrated in ducklings and pheasants in the absence of insect vectors.<sup>3,5,6,7</sup>

Although the significance of birds as reservoirs of Venezuelan equine encephalitis (VEE) virus, which is antigenically related to EEE virus, has not been established, blood virus titers high enough to infect mosquitoes have been found in cardinals and serum antibodies against VEE virus have been demonstrated in other birds.<sup>1,2,4</sup> The research reported here was initiated to study the capacity of ring-necked pheasants and chukars to harbor and transmit VEE virus.

### MATERIALS AND METHODS

Eight 6-month-old ring-necked pheasants which were serologically negative using the hemagglutination test (HI)<sup>8</sup> for VEE virus and EEE virus antibodies were obtained from a commercial source. Two males and two females were placed in two fibreglass cages, 1.2 x 0.9 x 0.9 m. One male and one female in each cage

were inoculated subcutaneously with  $10^8$  suckling mouse median lethal doses (SMLD<sub>50</sub>) of virulent VEE virus, strain GJ9-1BJ isolated from *Psorophora conformis* mosquitoes collected in Parcelamiento, Montufar, Guatemala in 1969 during an epizootic.<sup>1</sup> Four male and six female chukars were placed in a fibreglass cage 1.2 x 0.9 x 0.9 m and two males and three females were inoculated subcutaneously with  $10^8$  SMLD<sub>50</sub> of the same virus. The birds were observed daily for clinical signs.

Blood samples obtained from the jugular vein of each bird preinoculation and postinoculation on days 1, 3, 5, 7, 9, 11, 13, 16, 19, 22, 25, and 35, were placed in sterile glass tubes for serum collection. Collected serum was frozen at  $-60$  C until used. Cloacal swabs were taken each day the birds were bled and placed in 2 ml of brain heart infusion broth containing 5,000 units of penicillin, and 1,000  $\mu$ g streptomycin/ml. All birds were killed 35 days postinoculation. Half of the spleen, liver, kidney and brain were frozen at  $-60$  C for virus isolation and the other portion was placed in neutral buffered 10% formalin. Virus isolation was attempted on each serum, frozen tissue and cloacal swab sample using routine techniques.<sup>5</sup> A litter of eight suckling mice was inoculated intracerebrally with each sample, 0.2 ml/

<sup>1</sup> Obtained from CDC, Atlanta, GA.

mouse. Antibody titers in serum samples were determined using the HI test.<sup>5</sup> Sections were taken from the formalin fixed tissues, dehydrated in graded alcohols, embedded in paraffin, cut at 6  $\mu$ m and stained with hematoxylin and eosin.

## RESULTS

Clinical signs were not observed in inoculated or uninoculated pheasants or chukars. Viremia was detected in three of four inoculated pheasants on day 1 postinoculation. All suckling mice in the three litters inoculated with undiluted serum died within 7 days. In 1:10 dilutions of the same samples, deaths were not observed in suckling mice. A titer of  $10^{2.2}$  SMLD<sub>50</sub>/ml pheasant serum was calculated using Kaber's technique.<sup>8</sup> Viremia was also present in two of five inoculated chukars 3 days postinoculation in undiluted serum, but because the samples collected from chukars were small, titration was not attempted. Six of eight and seven of eight suckling mice died in respective litters within 7 days. Virus was

not detected in serum samples from uninoculated birds, tissue samples from all birds collected at necropsy, or in cloacal swabs. Microscopic lesions were not seen in tissues of infected birds.

Antibodies for VEE virus appeared in the serum of inoculated birds 7 days postinoculation. These HI antibody titers reached a peak of 1:1280 2 weeks postinoculation and gradually declined (Figure 1). Antibodies were not detected in uninoculated cage mates of pheasants or chukars.

## DISCUSSION

The absence of clinical signs indicates that this strain of VEE virus is not highly virulent for pheasants and chukars. This fact parallels previous results obtained when cardinals, sparrows, pigeons, and morning doves were infected with VEE virus.<sup>2</sup> Lack of clinical signs could represent a well-adapted, host-virus relationship which would contrast with the relationship of EEE virus and pheasants or chukars. In contrast, it could reflect only a partial resistance of an abnormal host to a virus with a broad host range. The absence of a high serum virus titer which lasts for several days in pheasants and in other birds infected with VEE virus does not support the hypothesis of a well adapted virus-bird relationship. Serum virus titers of  $10^{2.2}$ /ml in pheasants or of  $10^{2.1}$ /ml in other birds are well below titers of  $10^{7.5}$ /ml or greater observed in rodents.<sup>2</sup> Although mosquito infection was not attempted in pheasants, the serum virus titers were below the threshold  $10^{3.1}$ /ml necessary to infect *Aedes triseriatus*.<sup>2</sup> Virus was not excreted in the feces of infected birds as EEE virus is in infected pheasants.<sup>6</sup> The absence of serum antibodies in uninoculated cage mates also indicates that VEE virus does not spread from bird to bird in the absence of a vector.

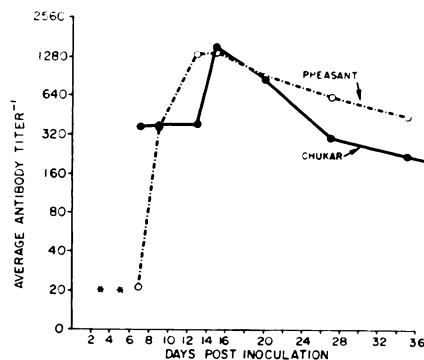


FIGURE 1. Average hemagglutination inhibition serum titers in four pheasants and five chukars inoculated with VEE virus. \*Samples were negative at the 1:10 dilution.

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