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Serum Antibody Prevalence for *Herpesvirus sylvilagus*, *Bacillus piliformis* and California Serogroup Arboviruses in Cottontail Rabbits from Pennsylvania

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ABSTRACT: A serologic survey of 60 eastern cottontail rabbits (*Sylvilagus floridanus*) from three counties in Pennsylvania was conducted in March 1983. Serum antibody prevalences for *Herpesvirus sylvilagus* and La Crosse virus (California serogroup) were <4%. There was no evidence of previous exposure to either Jamestown Canyon or snowshoe hare viruses (California serogroup). Antibody to trivittatus virus (California serogroup) was found in 60% of the 20 cottontails from York County. No cottontails had antibodies to *Bacillus piliformis*, the etiologic agent of Tyzzer's disease.

Key words: Cottontail, *Sylvilagus floridanus*, *Herpesvirus sylvilagus*, *Bacillus piliformis*, Tyzzer's disease, California group arboviruses, serology, serological survey.

Populations of the eastern cottontail rabbit (*Sylvilagus floridanus*) have been declining over portions of its original range (Edwards et al., 1981). Land-use changes have been recognized as a principal cause of these reductions at the regional level (Chapman et al., 1982). Many other factors such as disease, predation, adverse weather, dispersal, competition and nutritional inadequacies can decrease local populations (Chapman et al., 1982).

Herpesvirus sylvilagus, *Bacillus piliformis* (the etiologic agent of Tyzzer's disease), and California serogroup arboviruses have been reported in populations of cottontails (Ganaway et al., 1976; Lewis and Hinze, 1976; Seymour and Yuill, 1981). The epizootiology of *H. sylvilagus* in Wisconsin, the causative agent of a lymphoproliferative disease of the cottontail rabbit, was described by Lewis and Hinze (1976) and Spieker and Yuill (1976, 1977a, b) described various aspects of transmission of the virus. Tyzzer's disease in free-living cottontails from Maryland (Ganaway et al., 1976) is a contagious, fatal, enteric and hepatic disease caused by *B. piliformis*. La Crosse (LAC) and trivittatus (TVT) of the California serogroup arboviruses infect cottontails (Moulton and Thompson, 1971; Pinger et al., 1975). Little is known about the occurrence of these pathogens in cottontails from Pennsylvania. Thus, the objective of the present study was to determine the serum antibody prevalence to *H. sylvilagus*, *B. piliformis*, and California serogroup arboviruses in geographically separate populations of eastern cottontail rabbits from Pennsylvania.

In March 1983, 20 cottontails (10 adult males, 10 adult females) were live-trapped from several sites in each of three counties in Pennsylvania: Somerset (southcentral Pennsylvania, 39°57′N, 78°55′W), Westmoreland (southwestern, 40°20′N, 79°22′W), and York (southeastern, 40°5′N, 77°2′W). Cottontails from Somerset County were trapped from reclaimed strip-mined sites near Roxbury, Pennsylvania. Cottontails from Westmoreland County were trapped on farmland areas near Latrobe, Pennsylvania. Cottontails from York County were trapped on farmland and State Game Lands near Dillsburg, Pennsylvania.

Blood collection was by heart puncture with Vacutainer vacuum tubes (Becton, Dickinson and Company, Rutherford, New Jersey 07070, USA). Blood samples were

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allowed to clot for 30 min after collection, and then centrifuged at 1,000 *g* for 10 min. At least 0.5 ml of sera was immediately frozen at −10 C for shipment to the School of Veterinary Medicine, University of Wisconsin–Madison (Madison, Wisconsin 53706, USA) for *H. sylvilagus* and California serogroup arbovirus serological testing. An additional 0.5 ml of frozen sera was shipped to the National Institutes of Health (Bethesda, Maryland 20205, USA) for *B. piliformis* testing.

Antibody to *H. sylvilagus* was evaluated by 80% plaque reduction neutralization in tissue culture using methods described by Hinze (1971). All samples were tested at a dilution of 1:10.

B. piliformis originally was isolated from a laboratory rabbit (Ganaway et al., 1971). To prepare an enzyme-linked immunosorbent assay (ELISA) antigen, *B. piliformis* was grown in primary chicken embryonic liver cell cultures (Ganaway et al., 1985b). Details of the ELISA techniques were as published previously (Ganaway et al., 1985a).

Antibodies to LAC, TVT, snowshoe hare (SSH), and Jamestown Canyon (JC) California serogroup viruses were measured by microneutralization tests described by Pantuwatana et al. (1972) as modified by Ksiazek and Yuill (1977). Calculation of end points for virus titrations and log neutralization indices followed Reed and Muench (1938). Reference antibodies were hyperimmune mouse ascitic fluids prepared by the method of Brandt et al. (1967). Prepared virus stocks were LAC in the fifth suckling mouse brain (SMB) passage, TVT in the 17th SMB passage, SSH virus in the 23rd SMB passage, and JC in the sixth SMB passage (Ksiazek and Yuill, 1977). Titers <10 for each disease agent were considered negative.

Two rabbits, one adult female from Somerset County and one adult male from York County were positive for *H. sylvilagus*. Lewis and Hinze (1976) indicated that antibody prevalence was higher in adult males than in other sex and age groups. The low prevalence found in our study (<4%) suggests that *H. sylvilagus* was not a major source of mortality for cottontails in Pennsylvania during 1983.

Serologic evidence of SSH or JC was not found in any of the rabbits tested. Two adult male cottontails (10%) from Westmoreland County tested positive for LAC virus. La Crosse virus produces disease in humans and asymptomatic infection occurs in several small mammals, although not cottontails (Seymour and Yuill, 1981). Moulton and Thompson (1971) reported a 15% antibody prevalence for LAC virus in cottontails in Wisconsin. An earlier study in Pennsylvania reported LAC antibody prevalence in 254 cottontails to be <1% (Kradel et al., 1978). Our results fall between the values reported in these two studies.

One cottontail (5%) from Somerset County and twelve cottontails (60%) from York County tested positive for TVT virus. Titers ranged from 20 to 640. No sex specificity was evident for prevalence of antibodies to TVT virus. Neither LAC nor TVT cause clinical disease in cottontails but are maintained in cottontail populations and transmitted to other hosts by arthropods (Seymour and Yuill, 1981). Kradel et al. (1978) reported the prevalence of antibody to TVT was <1% of cottontails (n = 254) tested in central Pennsylvania. Pinger et al. (1975) reported 46% of cottontails (n = 22) from Iowa tested positive for TVT virus. Furthermore, they indicated a close association between the TVT virus vector, a mosquito (*Aedes trivittatus*), and the eastern cottontail. We are unable to explain the wide disparity in serum antibody prevalence for TVT virus between York County and the other collection areas. An attempt was not made to determine vector prevalence in the study area at the time blood samples were taken.

Serologic evidence of exposure to *B. piliformis* was not found in any of the 60 rabbits. Prevalence of Tyzzer's disease in free-ranging rabbit populations is not well known. Ganaway et al. (1976) hypothe-

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sized that this disease may be an important population controlling factor for cottontails because most exposed animals eventually succumb to the disease. Because we found no serologic evidence of *B. piliformis*, we concluded that Tyzzer's disease apparently has not occurred in these populations, antibody decay was rapid, or infected animals died.

Our results provide no evidence to support a contention that the selected diseases were widespread in the rabbit population of Pennsylvania during 1983.

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