Reports

Isolation of Colorado Tick Fever Virus from Rodents in Colorado

Although Colorado tick fever virus has been isolated frequently from man and from the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles (*1*), spontaneous infection of wild mammals has not been previously reported. Species expected to harbor the virus must necessarily be hosts of the immature stages of *D. andersoni,* the only tick that has been incriminated in transmission of the disease to man. We have obtained serologic evidence suggesting the occurrence of natural infections in one such animal species, the Columbian ground squirrel, *Citellus columbianus,* in western Montana and have found this species to be readily susceptible to experimental infection. This report records the first isolations of virus from mammals in nature. The hosts were the golden-mantled ground squirrel, *Citellus lateralis lateralis,* and the porcupine, *Erethizon dorsatum epixanthum,* collected in western Colorado.

Several members of a religious order residing on and operating a large ranch near Snowmass, Pitkin County, Colo., contracted Colorado tick fever in 1956 and 1957. Studies made 10–13 August 1957 revealed that golden-mantled ground squirrels occurred abundantly in pastures adjacent to the residence building and along roadsides on and near the ranch. Twenty were shot and four were captured alive. Two porcupines were captured about 2 miles from the residence. Other animals collected were one chipmunk, *Eutamias* sp., one deer mouse, *Peromyscus* sp., and one woodchuck, *Marmota flaviventris.* Blood samples were taken and refrigerated for transport to the laboratory. Fifteen adult *Dermacentor andersoni* were found on the porcupines, and 97 immature *D. andersoni* and 30 immature *Ixodes sculptus* on the squirrels. These and 33 adult *D. andersoni* collected from vegetation were saved for testing.

Blood samples were tested for presence of virus by triturating the clot in about 2 ml of physiologic saline solution, centrifuging the suspension at moderate speed, and injecting 0.05 ml of the supernatant intraperitoneally into each of a litter of six 4-day-old white mice. Transfers to passage mice were made by inoculation of brain suspensions.

Death resulted in four of six primary test mice injected with blood from one squirrel and in all passage mice. One mouse died in each litter injected with blood from another squirrel and from porcupine 34459, and several mice died in the passage litters. All three isolates were identified by neutralization tests as Colorado tick fever virus.

Blood samples collected 21 August from the four living ground squirrels were tested for virus and for neutralizing antibodies. No neutralizing antibodies were found, but virus was isolated from the blood of two of the animals, one of which had also been positive when it was bled in the field 7 days earlier. These four squirrels were then infested with virus-free laboratory-reared nymphs of *D. andersoni* to determine whether the blood of any of these animals contained sufficient virus to infect engorging ticks. One squirrel died before the ticks had opportunity to feed, but engorged ticks were obtained from the three remaining animals. When tested as adults, the ticks from one squirrel from which virus had been isolated earlier proved to be infected.

Serum samples from 20 ground squirrels, the deer mouse, the chipmunk, and porcupine 34459 did not contain neutralizing antibodies, but the sample from ground squirrel 34444, the woodchuck, and porcupine 34460 neutralized 264, 40, and 576 LD_{50} of Colorado tick fever virus, respectively.

The ticks obtained in the field were tested in separate lots according to host, species, and stage of tick—that is, larvae and nymphs from the same animal were tested separately. Ticks were triturated with sterile sand in physiologic saline solution containing 10 percent rabbit serum, streptomycin, and penicillin. Each sample was injected intraperitoneally into a litter of six 4-day-old mice. On the 8th day after inoculation, unless sickness was noted earlier, two mice of each litter were sacrificed and a brain suspension was passed to another litter. No infection was demonstrated in the adult ticks. By passage, virus was isolated from immature *D. andersoni* from two ground squirrels. One lot consisted of three larvae and the other of four nymphs. The host of the nymphs was one of the squirrels from which virus was isolated (*2*).

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References and Notes

1. C. M. Eklund, G. M. Kohls, J. M. Brennan, *J. Am. Med. Assoc.* 157, 335 (1955).
2. We are indebted to Dr. Gordon Meiklejohn, of the University of Colorado Medical School, for informing us of the occurrence of the Colorado tick fever cases at Snowmass and to Rev. M. Leo Slatterie for permission to collect animals and ticks on the monastery grounds.

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Taxonomic Implication of Actinophage Host-Range

Bacteriophages are known which attack hosts of different species and genera (*1*). However, bacterial viruses attack only closely related hosts. Therefore susceptibility to particular bacteriophages is used to speciate certain bacteria (*2*). Members of the genera *Nocardia* and *Streptomyces* are sensitive to their respective bacteriophages (*3*). Recently we found that two of 12 actinophages, initially isolated on streptomycetes, were able to attack some species of *Nocardia.* We therefore undertook to isolate additional streptomyces phages and nocardia phages in order to determine whether or not other intergeneric susceptibilities existed (*4*).

The strains of *Streptomyces* employed in this investigation have been previously described (*5*). The strains of *Nocardia* used, except for *Nocardia* sp. strain 3403, were supplied by Norman F. Conant, of Duke University. *Nocardia* sp. strain 3403 was obtained from the collection of the New Jersey Agricultural Experiment Station. The cultures of *Actinoplanes* and *Streptosporangium* were supplied by John Couch, of the University of North Carolina. The strains of *Micro-*

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