Kuns, M. L., and Robert Rausch 1950. An ecological study of helminths of some Wyoming voles. (*Microtus* spp.) with a description of a new species of *Nematospiroides* (Heligmosomidae: Nematoda). Zoologica 35: 181-188.

Otto, G. F. 1958. Some reflections on the ecology of parasitism. J. Parasitol. 44(1): 1-27.

Pearse, A. S. 1930. The ecology of the internal parasites of Nigerian rodents and insectivores. J. Elisha Mitchell Sci. Soc. 45(2): 221-228.

Rausch, Robert, and J. D. Tiner 1949. Studies on the parasitic helminths of the North Central States. II. Helminths of voles (*Microtus* spp.). Preliminary report, Amer. Midl. Natl. 41(3): 665-694.

PRESENT STATUS OF KNOWLEDGE ON THE ECOLOGY OF EASTERN ENCEPHALITIS VIRUS IN THE UNITED STATES

By DONALD D. STAMM

From the Communicable Disease Center, Public Health Service,
U. S. Department of Health, Education and Welfare, Box 61,
Montgomery, Ala.

THE DISEASE

The virus of eastern encephalitis (EE) produces highly fatal disease in humans, horses, and ring-necked pheasants (1-4). Outbreaks have occurred chiefly in states bordering the Atlantic and Gulf coasts. Less than 100 cases of human disease have been reported but mortality exceeds 60% and a large proportion of survivors are seriously and permanently disabled. Thousands of horses have been killed by the disease, usually in localized epidemics involving less than 100 cases; however, over 14,000 cases occurred in Louisiana in 1947. Mortality in horses is about 90% of the clinical cases. EE has produced many epidemics among captive ring-necked pheasants, with mortality of over 80% of involved flocks being common.

Epidemics have always occurred during summer months, chiefly in July, August, and September. In more southern areas they may begin as early as April.

It was suspected on epidemiological grounds as early as 1935 that wild birds were natural hosts and disseminators of the virus and that mosquitoes were natural vectors (5, 6). This suspicion has been amply substantiated by later work (7-11).

THE VIRUS IN NATURE

EE virus has been isolated from more than 20 species of naturally infected wild birds and specific antibody has been detected in more than 50 species. Field studies have shown wide spread of infection through wild bird populations during summer months (11-14). For example, in Massachusetts in 1956 and EE epidemic involved humans, horses, and ring-necked pheasants. EE virus was isolated from 3 of 152 wild birds collected in the affected area and antibody was present in 45%. During the same year in New Jersey, epidemics occurred in horses and ring-necked pheasants. EE virus was isolated from 7 of 143 birds and the antibody rate in resident birds increased from 14% in July to 54% in September. Other studies indicate comparable virus activity in Louisiana and Alabama.

Epidemics have usually occurred during hot, wet seasons when mosquito populations are high. Species of *Aedes, Mansonia,* and *Psorophora* mosquitoes are suspected of being epidemic vectors, but definite proof is lacking. There is strong evidence, however, that *Culiseta melanura* is the primary vector for bird-to-bird transmission of EE virus (8, 10). This species breeds in specialized areas of fresh water swamps and bogs (15). Seventeen virus isola-

tions have been made from *C. melanura* in four states. In New Jersey in 1956 an isolation was made for every 250 individuals of this species tested from the area for which bird infection data was presented above. Precipitin tests indicate that it feeds mainly on birds (16). How frequently it feeds on man or horses is not known.

Speculation on these bird and mosquito infection rates in New Jersey yields some interesting possibilities. We can assume a total population of all species and ages of birds of 2,000 per 100 acres during the last two weeks of August in the swampy woodland habitat studied. It is probable that half of this population are migrants and remain in the area for only one day. Five per cent of the bird population was observed to be circulating virus. Viremia is detectable for 4 days following infection, therefore one-fourth of this proportion, or 1.25% of the population, became infected each night. However, if we assume that half of the population left the area each day we only observed the virus in half the proportion infected each night. On this basis, 2.5%, or 1 in 40 birds present, became infected each night. Since 1 of 250 *C. melanura* in the area were infected, every bird present was bitten by about 6 of these mosquitoes each night. Knowledge of the numbers of mosquitoes which feed on each bird per night is as non existent as knowledge of true bird density and length of stay in such areas, but the figure does not seem improbable. The really important deduction is that 1.25% of the population, or 25 infected migrant birds, leave each 100 acres of this habitat daily. The potential for spread of the virus to other areas is thus very great since bird and mosquito populations will also be at a peak in these areas.

Hundreds of isolations of EE virus have been made from horses, and ring-necked pheasants and a few from humans when epidemics were in progress in habitat frequented by these species. Most of these epidemics have occurred in August and September. Studies designed to demonstrate the presence of virus when epidemics were not in progress have revealed it in a number of places during a much longer span of time (11-14, 17). In the specialized habitats in the southern United States where *C. melanura* is relatively abundant, EE virus has been isolated from birds or mosquitoes during all months from March through November, except September. Due to our anthropocentric viewpoint, we have not been observing EE virus activity in true proportion to its actual presence geographically and in time. Until human cases were observed in 1938 this was considered a horse disease only, as reflected in the name. Eastern Equine Encephalomyelitis employed until recently. Later investigations have shown that human and domestic animal disease is merely the above-surface fraction of the iceberg of its real activity in nature.

The method by which this virus survives through winter months is unknown. Overwintering in adult mosquitoes, latent infection in birds, and transovarian passage in mosquitoes have been postulated, but none have been proven. It is also conceivable that transmission may continue throughout the winter in some special southern habitats at rates reduced below those detectable by the effort so far expended. The tremendous volume of northbound migrants through southern areas in the spring could then reintroduce it to more northern areas.

LABORATORY STUDIES

More than 20 species of wild birds have been infected in the laboratory by subcutaneous inoculation of small amounts of virus (9). No species tested has proved refractory. After inoculation, the virus is usually present in the blood by the 18th hour, reaches peak concentration on the 2nd or 3rd day, recedes on the 4th day and is usually absent by the 5th. Sparrows, blackbirds and other small passerine species may circulate several thousand times as much virus as larger species such as herons and ibis. Specific antibody appears in the blood serum within a few days after viremia subsides and reaches a maximum level after about two months. So far as is known, birds are immune to reinfection for life. The mortality produced by EE in wild birds is difficult to assess. Laboratory studies indicate mortality may range from none to 80%, depending on species, dosage, and virus strain but it is not known whether this applies under natural conditions. It is probable, however, that mortality in

nestling birds is high since domestic chicken embryos and chicks up to 48 hours after hatching have been shown to suffer 100% mortality from minute amounts of virus (18). After chicks become 48 hours old, mortality is much lower, though virus circulates and they become immune.

Mosquitoes of a number of species have been fed on various concentrations of virus to determine the least amount that can infect them. By comparing these "infection thresholds" with viremia levels which occur in host species, gradients of host and vector potential have been obtained (19). While small birds can easily infect many species of mosquitoes, larger birds such as herons, ibis, and ring-necked pheasants, and horses rarely circulate sufficient virus to infect any but the most efficient species of mosquitoes. For these reasons, horses, ring-necked pheasants, and humans are usually considered dead ends in the natural transmission chain of EE.

UNANSWERED QUESTIONS AND DISCUSSION

The gaps in our knowledge of the ecology of EE virus and the epidemiology of the disease center on four questions. How does the virus survive through winter months? What ecological conditions usually confine virus activity to mosquitoes and birds in swampland habitat but periodically allow it to invade habitat where humans and domestic animals are present? Is it possible to predict these conditions or to detect them in their incipient stages? Finally, but most important, can involvement of humans and domestic animals be prevented?

In general the answers to all of these questions depend upon detailed quantitative knowledge of the density and movements of the host and vector populations.

As to the overwintering mechanism, it seems most probable that the answer lies in year-round activity, greatly reduced during the winter, in limited areas of the southern United States. Validation of this opinion will depend upon intensive studies in the suspect habitats.

In regard to the ecological conditions which produce epidemics of EE, it is generally accepted that relatively high mosquito populations are necessary. The relative density and movement of wild bird populations may be even more critical and has received little attention. Present methods of mosquito population estimation are very crude and information gained from their use is meagre. On the other hand, useful techniques for bird population estimation are quite well developed and have produced a great deal of valuable and dependable information (20, 21). Unfortunately, the bird census techniques generally employed are not well suited to the seasons and situations most critical in the study of EE, and relatively little pertinent information is available. EE epidemics occur at the end of the breeding season when bird populations are highest and when the maximum amount of movement is occurring due to post-nuptial and juvenile wandering and migration. Furthermore, epidemics occur most frequently near coastal areas where migrating birds are concentrated by wind and geographic factors. Odum has illustrated how natural habitat in the U. S. has been altered by human activity. "Man tends to create a forest edge habitat." The creation of increased edge and diversified habitat has a strong tendency to increase the number and density of species of many organisms and is especially marked in relation to bird populations. Bird populations average 144 per 100 acres in grassland, 465 in deciduous forests, and 827 in diversified man-made habitats. Extreme density may exceed 2,000 birds per 100 acres in the latter habitat (22). The continent-wide influence of settlement and introduction of foreign species may have increased considerably the total numbers of birds present. Undoubtedly it has greatly multiplied the numbers in close proximity to human habitation. Under these altered conditions the coincidence of a series of good breeding years may increase populations of birds and mosquitoes to a point where natural controls on transmission of any disease agent break down. This trend towards change in habitat is still very much in effect. Urban and suburban residential areas and highway and other rights-of-way are expanding rapidly and will continue to do so parallel with human population growth (23). It it necessary to know what the effect of such trends will be on bird populations in order to assess the future epidemic potential of diseases such as EE. Most bird census studies have been made employing the spot-mapping technique (21). This method produces good results during breeding and winter seasons but it is not adapted

to use in late summer when a great deal of bird movement is going on. Recently a beginning has been made in developing a bird population estimation method which may overcome these difficulties (24). Birds are captured in mist nets, banded, and released and the population is calculated on the basis of the proportion of banded birds in a later sample. The method is especially well adapted to following activity of agents such as EE virus in bird populations. Large numbers of birds can be tested with little disturbance of the population, and a measure can be obtained of current virus activity as well as that occurring between successive periods of study.

If this hypothesis of the critical nature of bird density and movement in the epidemiology of EE can be substantiated, then prediction of danger can be based on bird population estimation. This possibility can only be evaluated by following the trend in bird density for a number of years in areas such as Massachusetts where epidemics have occurred repeatedly. Such studies must be continuous during critical seasons, since bird density and distribution change on a day-to-day basis at such times.

The presence of introduced pest species probably greatly increases the danger to man from diseases with avian reservoirs. House sparrows, starlings, and domestic pigeons increase bird density in man's immediate environment and serve as a link between this and more natural habitats.

Methods of prevention and control conceivable at present could involve reduction in density of mosquitoes or birds, or both. *Culiseta melanura* breeds in a specialized habitat. An attack concentrated on this habitat should reduce the extent and numbers of endemic foci, especially in areas where human disease has occurred. Every possible effort should be made to eliminate pest species of birds. This alone might reduce total bird density enough near human habitation to decrease the problem.

Methods of rapid simultaneous reduction of both mosquito and bird populations should be available for emergency use. It is critical that such techniques be strictly limited in the area affected and the duration of the effect. It would probably be necessary to employ such methods only for short periods in limited areas, and if the above criteria are met no important damage to desirable species should result. Such techniques must be fully developed and evaluated before emergencies occur, since an epidemic situation is not conducive to clear-headedness. The best defense for those people concerned with protection of wildlife against uncritical and unwise activity of this sort will be to have developed sensible methods ahead of time.

REFERENCES

1. Feemster, R. F. 1957. Equine Encephalitis in Massachusetts. New Eng. J. Med. 257:701-704.
2. Howitt, B. F., L. K. Bishop, R. H. Gorris, R. E. Kissling, G. H. Hauser and W. L. Treuting. 1948. An Outbreak of Equine Encephalomyelitis, Eastern Type, in Southwestern Louisiana. Proc. Soc. Exper. Biol. and Med. 68:70-72.
3. Beadle, L. D. 1959. Status of Mosquito-Borne Encephalitis in the United States. Pub. Health Rep. 74:84-90.
4. Ferguson, F. F. 1954. Biological Factors in the Transmission of Arthropod-Borne Encephalitides. Public Health Monograph No. 23.
5. TenBroeck, C., E. W. Hurst and E. Traub. 1935. Epidemiology of Equine Encephalomyelitis in the Eastern United States. Jour. Exper. Med. 62:677-685.
6. TenBroeck, C. 1938. Birds as Possible Carriers of the Virus of Equine Encephalomyelitis. Arch. Pathol. 25:279.
7. Davis, W. A. 1940. A Study of Birds and Mosquitoes as Hosts for the Virus of Eastern Equine Encephalomyelitis. Amer. Jour. Hyg. 32(Sect. C):45-49.
8. Chamberlain, R. W. 1958. Vector Relationships of the Arthropod-Borne Encephalitides in North America. Ann. N. Y. Acad. Sci. 70 (Art. 3): 312-319.

9. Kissling, R. E. 1958. Host Relationships of the Arthropod-Borne Encephalitides. Ann. N. Y. Acad. Sci. 70 (Art. 3) :320-327.
10. Hess, A. D. and P. Holden. 1958. The Natural History of the Arthropod-Borne Encephalitides in the United States. Ann. N. Y. Acad. Sci. 70 (Art. 3) :294-311.
11. Stamm, D. D. 1958. Studies on the Ecology of Equine Encephalomyelitis. Amer. Jour. Pub. Health 48 :328-335.
12. Kissling, R. E., R. W. Chamberlain, D. B. Nelson and D. D. Stamm. 1955. Studies on the North American Arthropod-Borne Encephalitides. VIII. Equine Encephalitis Studies in Louisiana. Amer. Jour. Hyg. 62 :233-254.
13. Kissling, R. E., D. D. Stamm, R. W. Chamberlain and W. D. Sudia. 1957. Birds as Winter Hosts for Eastern and Western Equine Encephalomyelitis Viruses. Amer. Jour. Hyg. 66 :42-47.
14. Feemster, R. F., R. E. Wheeler, J. B. Daniels, H. D. Rose, M. Schaeffer, R. E. Kissling, R. O. Hayes, E. R. Alexander and W. A. Murray. 1958. Field and Laboratory Studies on Equine Encephalitis. New Eng. Jour. Med. 259 :107-113.
15. Burbutis, P. P. and R. W. Lake. 1956. The Biology of *Culiseta melanura* (Coquillet) in New Jersey. Proc. 43rd Annual Meeting, New Jersey Mosquito Extermination Association, Atlantic City, N. J. pp. 155-161.
16. Burbutis, P. P. and D. M. Jobbins. 1957. *Culiseta melanura* Coq. and Eastern Equine Encephalomyelitis in New Jersey. Proc. 44th Annual Meeting, New Jersey Mosquito Extermination Association, New Brunswick, N. J. pp. 68-78.
17. Stamm, D. D. Unpublished Data.
18. Chamberlain, R. W., R. K. Sikes and R. E. Kissling. 1954. Use of Chicks in Eastern and Western Equine Encephalitis Studies. J. Immunol. 73 :106-114.
19. Schaeffer, M. and E. H. Arnold. 1954. Studies on the North American Arthropod-Borne Encephalitides. I. Introduction. Contributions of Newer Field-Laboratory Approaches. Amer. Jour. Hyg. 60 :231-236.
20. Kendeigh, S. C. 1944. Measurement of Bird Populations. Ecol. Mono. 14, pp. 67-106.
21. Audubon Field Notes 4 :185, 1950.
22. Odum, E. P. 1959. Fundamentals of Ecology, 2nd Ed. W. B. Saunders Co., Philadelphia and London.
23. Scott, T. G. 1958. The Ornithologists Responsibility to the Future. A Contribution from the Wilson Ornithological Society Conservation Committee. Wilson Bulletin 70 :385-393.
24. Stamm, D. D., D. E. Davis and C. S. Robbins. A Method of Studying Wild Bird Populations by Mist-Netting and Banding. To be Published.