The virus of eastern equine encephalomyelitis seems well established in the eastern United States. Eradication is probably impossible or impractical. It is necessary therefore to understand the ecologic balances which permit the EEE virus to survive or to produce an epidemic situation. The present study summarizes a number of field investigations in Louisiana, New Jersey, Massachusetts, and Alabama to determine the existence and spread of equine encephalomyelitis viruses.

STUDIES ON THE ECOLOGY OF EQUINE ENCEPHALOMYELITIS

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EVIDENCE OF repeated activity of eastern (EEE) and western (WEE) equine encephalomyelitis virus among wild birds in several areas of the eastern United States has been reported by Kissling, et al.[1, 2] The same workers have also demonstrated the susceptibility of birds to these viruses in the laboratory and measured their potential as sources of virus for mosquito infection.[1, 3] A year-round search for virus and study of antibody patterns in wild birds in Louisiana indicated that virus activity occurred at a very low level or was absent during winter months.[2] Serologic evidence also suggested that migratory birds were undergoing additional exposure during their sojourn south of the United States. This evidence, together with data on duration of viremia, suggested the possibility of reintroduction of virus by migratory birds each spring. However, studies designed to check this hypothesis did not support it and, in fact, pointed even more strongly to fresh water swamps as the site of the permanent reservoir for EEE and WEE in the eastern United States.[4]

The present paper summarizes a series of field investigations which constitute a continuation and geographic extension of the above studies. The data consist largely of the results of virus isolation and serum neutralization tests on wild bird blood collected in Louisiana, New Jersey, and Massachusetts. For purposes of comparison, published and unpublished data of the writer and other workers are included.

The technics of collecting and handling specimens and laboratory procedures were similar to those reported in previous studies,[2] except as noted.

Studies in Louisiana in 1956

In 1952–1953, 80 per cent of the yellow-crowned night herons * (YCNH) tested were found to have antibody to EEE, the highest antibody rate of any species collected in significant numbers [2]

* The names of bird species included in this paper follow the "Check-list of North American Birds" (5th ed.), prepared by a committee of the American Ornithologists Union, 1957.

(Table 1). As an explanation of the high rate of exposure of this species might suggest new areas for investigation, the following study was made in 1956.

A sample of 50 adult YCNH was collected in March, 1956, within a week after their return from South and Central America. Ninety per cent of these birds were found to be immune to EEE and 57 per cent to WEE. During the last two weeks in May, 189 nestling YCNH were bled and banded; 101 of these were re-bled a week later, and 33 were bled for the third time two weeks after the first bleeding. EEE virus was isolated from the blood of one of these nestling YCNH (Table 2) and seven

Table 1—Eastern and Western Equine Encephalomyelitis Serum Neutralizing Antibody in Birds

Source of Sample	Species	Date Collected		Positive EEE		Positive WEE	
		Year	Month	Positive/ tested	Per cent	Positive/ tested	Per cent
Louisiana	Red-winged blackbird Cardinal Brown thrasher Mocking-bird	1952–1953	Apr. through June	5/29	17	2/26	8
		1956	Mar.	13/24	54	2/24	8
			Sept.	4/31	13	1/31	3
	Yellow-crowned night heron	1952–1953	Apr. through June	24/30	80	7/27	25
		1956	Mar.	44/49	90	28/49	57
	Green heron Louisiana heron Little blue heron	1952–1953	Apr. through June	4/30	13	4/29	14
		1956	Sept.	2/26	8	0/16	0
New Jersey (Mays Landing)	Resident	1956	July	11/85	13	1/84	1
			Aug.–Sept.	31/57	54	4/57	7
Massachusetts	All species	1953	July–Sept.	32/146	22	18/142	13
		1956	Aug.–Sept.	67/149	45	0/146	0
Alabama	Resident	1957	Mar.	4/25	16		
			Aug.	15/52	29		
			Sept.	11/24	46		

others showed a conversion from negative to positive or a significant rise in titer in neutralization tests. Six weeks later and within 100 yards of the spot at which the virus-infected YCNH blood sample was obtained, 37 Louisiana heron and little blue heron nestlings were bled. Only two of these samples neutralized EEE virus. These observations implied that even though the virus was present, little spread of EEE took place during this period.

Table 2—Isolations of Eastern and Western Equine Encephalomyelitis Virus from Naturally Infected Birds

Source	Year	Month	Bird Species	Virus
Louisiana	1950	June	Purple grackle	EEE
	1952	Apr.	Catbird	EEE
		June	Loggerhead shrike	WEE
			Carolina chickadee	WEE
			Cardinal	WEE
		July	Cardinal	EEE
	1953	Mar.	Hermit thrush	EEE
	1956	Mar.	Mockingbird	EEE
		May	Purple grackle	WEE
			Yellow-crowned night heron	EEE
Massachusetts	1953	Sept.	Catbird	EEE
	1956	Aug.	English sparrow	EEE
			Lesser yellowlegs	EEE
		Sept.	Domestic pigeon	EEE (twice)
New Jersey	1956	Aug. 13 through 16	Carolina chickadee	EEE
			Eastern kingbird	EEE
			English sparrow	WEE (twice)
			Yellow-throated vireo	EEE
			" " "	WEE
		Aug. 30 through Sept. 5	Carolina chickadee	EEE (twice)
			Crow	WEE
			Carolina wren	EEE
			Catbird	EEE
			Eastern phoebe	WEE
			English sparrow	EEE
			Northern waterthrush	EEE
			Red-eyed vireo	WEE
			Song sparrow	EEE
Alabama	1957	July	White-eyed vireo	EEE
			Cardinal	EEE
		Aug.	Kentucky warbler	EEE

It was planned to assess virus activity in the immature YCNH during July and August on the basis of a sample collected in September. No individuals of this species were observed in the area during the first week in September, therefore the information was not obtainable. The high rate of antibody in the adult population is, however, at least partially explainable on the basis of other observations. First, the age of the breeding population of this species is at least three years, permitting exposure over several seasons. (The age of the breeding population of smaller passerine species, on the other hand, is slightly in excess of one year.) Next, this species nests only on the edges of permanent deep holes of water in this area. These sites are also the favored breeding place for Culiseta melanura, a species of mosquito which appears to be especially implicated in the ecology of EEE.[5] This close association with an important vector probably increases exposure to infection. Peculiarities of behavior may increase the exposure of these birds to mosquito bite. This species is very quiet and secretive and will stand motionless for long periods when disturbed. YCNH nestlings were observed to stand quietly and allow scores of mosquitoes to feed on them while little blue heron, green heron, and Louisiana heron nestlings in adjacent nests actively resisted mosquitoes and drove them away.

Other species of wild birds in the area were sampled during 1956 to assess current virus activity and allow comparison with data from preceding years. EEE virus was isolated from a mockingbird collected in March and WEE virus from a purple grackle collected in May. These isolations and all preceding and subsequent isolations of EEE and WEE virus from naturally infected wild birds made at this laboratory to date are listed in Table 2.

Results of neutralization studies on small passerine species (red-winged blackbird, cardinal, mockingbird, brown thrasher) and herons (green heron, Louisiana heron, little blue heron) collected in 1956 are shown in Table 1, along with data from the 1952–1953 study [2] for comparison. The data listed represent comparable samples from a given area, considering the flight range of individual bird species. All individuals of the small passerine species listed were collected along the same two and a half miles of railroad through Manchac swamp while the herons were collected throughout the swamp.

The EEE antibody rate of 17 per cent in the small passerine species, observed in the 1952–1953 study, increased to 54 per cent in the March, 1956, collection and dropped to 13 per cent in the September collection. This trend suggests that EEE virus was very active in the area during the summer of 1955 and that the high level of immunity in the spring of 1956 suppressed its activity during the subsequent summer months. The WEE antibody rates were identical at 8 per cent for the first two periods compared and somewhat lower in the September, 1956, sample; this is possibly the result of suppression of WEE activity by the high rate of EEE immunity.[6] EEE antibody rates in herons other than the YCNH did not vary greatly between the 1952–1953 and 1956 samples while WEE antibody was absent from the latter sample (Table 1).

Studies in New Jersey in 1956

As an extension of studies on EEE and WEE initiated in New Jersey by Holden, et al., in 1953,[7] wild birds were collected in two different areas during the summer of 1956.[8] Epidemics of EEE in ring-necked pheasants and horses occurred concurrently and were investigated by Sussman and Cohen.[9]

The first area from which birds were collected was the vicinity of a pheasant farm near Mays Landing, N. J. Epi-

demics of EEE in ring-necked pheasants had occurred repeatedly on this farm in previous years and both EEE and WEE viruses were isolated from wild birds collected on the premises by Holden in 1953. No virus was isolated from 109 wild birds collected during the first week of July, 1956. WEE virus was isolated from two English sparrows of a sample of 12 birds collected in this area on August 13, and EEE virus from a Carolina chickadee and a yellow-throated vireo of a sample of 19 collected on August 15. On August 16, 13 birds were collected and EEE virus was isolated from one eastern kingbird and WEE virus from a yellow-throated vireo. The next collections in this area were begun on August 30; on this date EEE virus was isolated from a Carolina chickadee of a sample of 20 birds. On August 31, an isolation of EEE virus was made from a Carolina wren and WEE was isolated from a red-eyed vireo of a sample of 31 birds. On September 1, EEE virus was isolated from a northern water-thrush of a sample of 28 birds. Twenty-four birds were collected on September 2 and yielded one isolation of EEE virus from a Carolina chickadee and one isolation of WEE virus from an eastern phoebe. One American crow, of three birds collected on September 3, yielded WEE virus. No virus was isolated from a sample of 54 birds collected in the same area during November.

The extent of activity of these viruses in this area as reflected by serum neutralization tests is shown in Table 1. Data are shown for 17 species considered to represent a geographically stable population, individuals of which were collected in both the July, and August 30 through September 3 collections. Of this sample 13 per cent were positive to EEE in July and 54 per cent were positive in the August-September collection. The WEE antibody rate increased from one per cent in July to seven per cent in the August-September collection (Table 1). The moderate level of immunity in the bird population at Mays Landing in July, 1956, is compatible with activity of the viruses at a maintenance level in an endemic swampland habitat. A violent burst of virus activity then occurred, resulting in the very high level of EEE immunity observed in the August-September sample.

The second area from which birds were collected was in the vicinity of a pheasant farm at Deans, N. J. An epidemic of EEE in ring-necked pheasants had been in progress on this farm for a week when the collections were made. On September 4, 18 wild birds collected within half a mile of the pheasant pens yielded two isolations of EEE virus, one from a catbird and one from a song sparrow. The next day 16 additional birds were collected and EEE virus was isolated from an English sparrow of this lot. When serum neutralization tests were run on these samples, only one was positive to EEE and none to WEE. The high percentage of isolations at Deans indicates that the virus was spreading through the wild bird population at a very high rate. The very low antibody level suggests that the virus had not been present the previous summer and had only recently appeared in this area.

Studies in Massachusetts

In 1953 weather conditions during the spring months were observed by Feemster to be similar to those which preceded the EEE epidemic of 1938.[10] To assess the current threat a sample of 156 birds was collected from the Hockamock swamp which appeared to have been the point of origin of the 1938 epidemic. EEE virus was isolated from a catbird of this sample and on serum neutralization tests 22 per cent were positive to EEE and 13 per cent to WEE [11] (Table 1). No human cases

and only two equine cases of EEE were recorded in Massachusetts during the summer of 1953.

During 1956 an epidemic of EEE involved human beings, horses, and ring-necked pheasants in Massachusetts. Between August 30 and September 9, 154 birds were collected from the vicinity of the Hockamock swamp.[11] Of this sample, two domestic pigeons, one English sparrow, and one lesser yellowlegs yielded EEE virus (Table 2). The sparrow and the yellowlegs were part of a collection of 18 birds made on August 31 in one locality. Serum neutralization tests on this sample revealed that 45 per cent of the birds were immune to EEE but none to WEE (Table 1).

The rate of isolations and immunity level observed here during 1953 again suggest an endemic situation, while the findings in 1956 fit an epidemic pattern and closely parallel concurrent observations in New Jersey.

Studies in Alabama in 1957

As the preceding studies indicated a need for more detailed knowledge of those factors that control the rate of spread of these viruses and limit it geographically, an intensive study of the activity of these viruses in a limited area of endemic habitat was begun.

A farm in Baldwin County, Ala., was chosen as a site for these investigations. The history of the area indicates that EEE virus has been established there for many years. A heavy bird population is present and a preliminary survey revealed a 16 per cent rate of EEE immunity. An entomologic survey indicated that breeding populations of a number of efficient vectors are present, including Culiseta melanura. It is planned to quantitate all observable factors in the transmission of the virus through the bird population by monthly sampling during at least one year. Entomologic studies are being made concurrently. With this approach it may be possible to define the mechanism of conversion of virus activity from endemic status in a sylvan habitat to epidemic spread outside of its usual geographic limits.

The preliminary sample of birds from this area was obtained by shooting; since then the birds have been captured in nets, banded, bled from the jugular vein, and released. The use of Japanese mist nets described by Austin [12] and Low [13] has proved to be a very efficient method of sampling birds for these purposes. This method of collection causes very little disturbance of the bird population as compared to shooting. The use of numbered bands also makes it possible to estimate the number of birds present, the age composition, and fluctuations in density of the population. To date, the rate of recapture has been very encouraging and will permit calculation of conversion rates on serologic tests.

Birds have been netted and bled each month from May through September. EEE virus was recovered from a white-eyed vireo of a sample of 25 birds bled on July 31, from a cardinal of 15 birds bled August 2, and from a Kentucky warbler of 40 birds bled on August 28. No virus was isolated from 106 birds collected on September 24 through 26.

EEE neutralizing antibody was present in 16 per cent of the survey sample collected in March, in 29 per cent of a sample collected on August 27 through 29, and had increased to 46 per cent in the September 24 through 26 collection. These data include only birds considered to be resident in the area during the period spanned; transients and winter residents are excluded.

This high rate of virus recovery and increase in antibody rate parallel the observations in New Jersey and Massachusetts in 1956. An epidemic of EEE in horses occurred in Alabama during the summer of 1957, with cases occur-

ring in areas of the state from which none had been reported for at least 10 years.

Discussion

When the results of studies made on EEE in wild birds in different localities over the course of several years are summarized, two differing patterns of activity appear to prevail.

The first pattern appears to be the progression of the virus through a wild bird population at a normal endemic maintenance rate. This appears to have been the case in Louisiana in 1952, 1953, and 1956, and in Massachusetts in 1953. It also presumably was the case in New Jersey in 1955 and in Alabama in 1956 as indicated by the residual immunity rates observed early the following year. Under these conditions it was possible to isolate virus from less than one per cent of the birds collected and 13–22 per cent of the population were found to possess neutralizing antibody. Very little or no human or horse involvement occurred in any of these areas during the years mentioned. Such a level of activity would seem to favor the continued presence of the virus.

A second pattern of activity of EEE virus in wild birds was observed in two different localities in New Jersey and also in Massachusetts during 1956 and in Alabama in 1957. It may also be presumed to have occurred in Louisiana in 1955 on the basis of the 54 per cent immunity rate observed in the March, 1956, collection. A similar situation appears to have been present in New Jersey in 1953.[7] On these occasions the virus seems to have spread through the wild bird population with explosive speed. Bird species such as English sparrows and domestic pigeons which are not involved in the endemic sylvan cycle became involved. On several occasions virus was isolated from as high as 11 per cent of the birds in a day's collection and this activity was seen to result in the immunity of 45–54 per cent of the population. Such a level of immunity would seem to jeopardize the continued presence of the virus. The virus also appeared outside of its usual geographic limits and on all of the instances mentioned equines were involved in epidemic proportions. Human cases of EEE occurred in Louisiana in 1955 and in Massachusetts in 1956. EEE epidemics also occurred in ring-necked pheasants in New Jersey in 1953 and 1956 and in Massachusetts in 1956.

Such hyperactivity also seems to have occurred with WEE virus in Louisiana in 1952 [2] and in New Jersey in 1956. However, the over-all rate of activity of WEE in the eastern United States seems to be lower than that of EEE, minimizing the importance of such occurrences proportionately.

The ecologic balances which hold the activity of EEE virus to a level compatible with its survival in appropriate areas are not well understood. However, it seems logical to conclude that its maintenance depends on sufficiently susceptible bird populations of appropriate density and upon a vector population of proper transmitting efficiency and also at an optimum density. These balances may be upset, conceivably by great increases in numbers of either birds or the usual vectors. It is more probable, however, that it is the entrance into the transmission cycle of other highly efficient mosquito species in large numbers which produces an epidemic situation.[5]

Conditions seem to be well established for the maintenance of these viruses in the eastern United States and to be of such long-standing and wide occurrence that their eradication from nature is probably impossible or impractical. On the other hand, an understanding of the factors responsible for these sudden, presumably abnormal bursts of virus activity may enable us to predict epidemics,

or detect them in their incipient stages, and possibly prevent the involvement of man or domestic animals.

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This paper was presented before a Joint Session of the Conference of Public Health Veterinarians and the Laboratory Section of the American Public Health Association at the Eighty-Fifth Annual Meeting in Cleveland, Ohio, November 13, 1957.

Simplicity in Science

Nature appears as a complex system whose factors are dimly discerned by us. But, as I ask you, Is not this the very truth? Should we not distrust the jaunty assurance with which every age prides itself that it at last has hit upon the ultimate concepts in which all that happens can be formulated? The aim of science is to seek the simplest explanations of complex facts. We are apt to fall into the error of thinking that the facts are simple because simplicity is the goal of our quest. The guiding motto in the life of every natural philosopher should be, Seek simplicity and distrust it.—Alfred North Whitehead, "The Concept of Nature"