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EPIDEMIOLOGY OF *PLASMODIUM HEXAMERIUM* HUFF, 1935, IN MEADOWLARKS AND STARLINGS OF THE CHEYENNE BOTTOMS, BARTON COUNTY, KANSAS*

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ABSTRACT: A 2-year epidemiological study of *Plasmodium hexamerium* in meadowlarks and starlings of the Cheyenne Bottoms Waterfowl Management Area, Barton County, Kansas, revealed that seasonal infection incidence varied significantly in the meadowlark, being highest in the early spring and lowest in the winter, but did not vary significantly in the starling. Both host species became infected at an early age, the meadowlark in the nest and the starling soon after leaving it. In concurrent vector studies: (1) *Culex tarsalis* was infected with *P. hexamerium* in the laboratory; (2) *C. tarsalis, Aedes nigromaculis,* and *A. sollicitans* were exceedingly abundant and fed regularly on small birds in the field, therefore all are considered potential vectors with *C. tarsalis* the most likely; (3) mosquito populations were not high until June when annual transmission probably begins; (4) populations of probable vectors are too low during March and April to account for the observed increase in malaria incidence in meadowlarks collected at that time. Overall differences in the epidemiological patterns of *P. hexamerium* in the meadowlark and starling are credited principally to differences in migratory habitats of the two birds and degree of ecological association with vectors.

The natural transmission of avian malaria is one of the most poorly understood aspects of the biology of *Plasmodium*. Herman's (1938) study of malaria in the redwing (*Agelaius phoeniceus* (L.)) is a classic and has been supplemented only recently by the work of Fallis and others on *Leucocytozoon* (Fallis, Anderson, and Bennett, 1956; Fallis and Bennett, 1963; Fallis, Davies, and Vickers, 1951; Fallis, Pearson, and Bennett, 1954). Growing interest in the ecology of the encephalitis viruses, along with the suggestion that bird malaria studies may shed light on this problem (Herman et al., 1954), makes further work on the epidemiology of avian malaria desirable. The present survey was part of a general study of animal parasites and viruses in central Kansas which began in February 1963 and is still in progress.

The meadowlarks (*Sturnella magna* (L.) and *S. neglecta* Aud.) and European starling (*Sturnus vulgaris* L.) were studied because both were found to harbor *Plasmodium hexamerium* Huff. Thus, variations in epidemiological patterns of this parasite in its different hosts might be manifestations of factors which generally affect avian malaria epidemiology.

THE STUDY AREA

The Cheyenne Bottoms Waterfowl Management Area is a prairie marsh located near Great Bend, Kansas. It is a sinkhole of approximately 30 square miles' area. Water is supplied by Walnut Creek and is maintained by dikes, gates, and canals at a depth of 2 to 3 ft deep except when certain pools are allowed to drain for management purposes. Cattails (*Typha latifolia* L.), sawgrass (*Scirpus fluviatilis* Gray), sedge (*Eleocharis macrostachya* Britt.), and Japanese millet (planted) are the principal marsh grasses, and a dense cattail growth covers much of the permanent water area. The outlying edges of the marsh merge with pastures and cultivated fields and extensive shallow flooding of these areas occurs following heavy spring rains. The nearest trees are several stands of cottonwoods within 2 or 3 miles of the water's edge.

Rainfall is usually heaviest in late spring and early fall. Summer is typically dry with extended periods of daytime temperatures over 100 F.

The area is a major migration resting center and nesting ground for waterfowl, shorebirds, and blackbirds.

The wildlife management, public hunting,

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and refuge activities are under the control of the Kansas Forestry, Fish, and Game Commission.

METHODS

The majority of blood smears studied were from shot-killed birds, though some starling smears were from live captives or nestlings. The latter were kept for 10 days in the laboratory, then a series of blood smears taken on alternate days was examined to determine if the birds were infected at the time of removal from the nest. Smears from heart, lung, or other tissues of shot birds were made in the field as quickly as possible after collection. Blood and tissue smears were stained in Giemsa and examined for at least 10 min under oil immersion at 1,000 ×.

Seasons were arbitrarily established as follows: summer, June–August; fall, September–November; winter, December–February; spring, March–May. Seasonal incidence was calculated on the basis of these criteria.

The starling strain of *Plasmodium hexamerium* was isolated from a bird captured in January 1964, and was maintained in wild-caught adults and laboratory-reared nestlings by subinoculation of infected blood. One-fourth cubic centimeter of a mixture of 0.75 ml blood and 0.25 ml saline–citrate was injected intravenously (jugular) to transfer infections.

Mosquito collections were made from March through October of two consecutive years. Adults were captured by New Jersey light traps, both battery and 110V operated, dry ice traps, baited traps, sweep nets, and aspirators. Adults were kept frozen until the time of identification, sometimes several weeks later. Mosquitoes were identified with the keys of Carpenter and LaCasse (1955). Adults reared from wild-caught larvae were used for infectivity studies. These were fed daily on sugar water except when attempts were made to feed them on birds. Mosquitoes fed on infected birds were examined for oocysts after 10 days and for sporozoites at varying intervals thereafter.

RESULTS

The seasonal incidence of *P. hexamerium* infections in the starling and meadowlark is shown in Figure 1. Contingency table analyses of these data (Dixon and Massey, 1957) indicate that the infection rate is independent of the season at the 5% level in the starling, but is not independent of season in the meadowlark (*P*/independence/less than 0.005).

Age incidence data are shown in Table I. "Nestlings" are birds taken from the nest at the age of 3 or 4 days. "Fledglings" are birds which, by their coloration and/or feather characteristics could be identified as young of the year. All "fledglings" were 1 to 2 months old.

TABLE I. P. hexamerium *in "young of the year" birds.*

	Starling	Meadowlark
Nestling		
Collected	18	8
Infected	0	1
Fledgling		
Collected	12	10
Infected	3	4

Characteristics of the parasite

Parasites identified as *P. hexamerium* exhibited minor differences in different hosts. The number of nuclei in apparently mature circulating segmenters tended to be higher in the starling (usually six) than in the meadowlark (usually four). Gametocytes in the starling were larger and more elongate than those in the meadowlark. Pigmentation was light in both parasites, particularly in the asexual stages where one or two pigment granules was typical. The undifferentiated trophozoites and immature gametocytes in the starling usually lay close to the nucleus of the host cell while those of the meadowlark tended to lie free toward the end of the cell.

The course of laboratory infections transmitted by subinoculation to starlings conformed most closely to that of avian malaria of low virulence as summarized by Hewitt (1940). Parasitemia in these infections never exceeded 60 parasites/10,000 erythrocytes. Circulating parasites did not appear until 15 to 29 days after subinoculation in four laboratory-reared birds and could be seen in the blood of two of these birds for up to 5 months after onset of patency. The parasite also proved noninfective for nine English sparrows (*Passer domesticus* L.) and three 3-day-old Pekin ducks.

Ornithological observations

Starlings remained principally in areas more than a mile from the water's edge, frequenting fields and feedlots during the day and roosting high in barns or trees, depending on the season, at night. Important nesting localities were cottonwood stands near the marsh. Nesting began in April and occurred typically in tree holes up to 60 ft high. After fledging, young of the year moved to fields in small flocks, possibly family groups, during the day and returned to the trees at night.

TABLE II. *Summary of adult mosquito collections.*

	Number captured*								Number of times captured
	April	May	June	July	Aug.	Sept.	Oct.	Total	
Culex tarsalis	0	73	703	475	2,016	1,850	344	5,461	59
Culex pipiens	0	2	55	1	76	112	14	260	24
Culiseta inornata	77	11	3	1	1	9	457	560	21
Aedes dorsalis	38	22	1,637	1,237	46	538	61	3,579	38
Aedes vexans	0	2	666	267	74	111	105	1,225	34
Aedes nigromaculis	0	7	1,312	163	84	136	29	1,731	35
Aedes sollicitans	0	1	1,274	56	22	22	7	1,382	30
Psorophora cyanescens	0	0	2	5	0	0	0	7	4
Psorophora ciliata	0	0	24	2	0	2	0	28	9
Orthopodomyia signifera	0	0	0	0	0	0	1	1	1
Anopheles punctipennis	0	0	0	0	0	42	0	42	3
Total	115	118	5,676	2,207	2,319	2,822	1,018	14,276	

* No mosquitoes captured during March or November although trapping activities were continuous.

Meadowlarks occurred in fairly large numbers throughout all of the area bordering the marsh and along dike roads. Important nesting areas were open fields north and south of the marsh. All of the important nesting areas were also those localities in which floodwater mosquitoes were most numerous.

Vectors

Eleven species of mosquitoes were identified from the Cheyenne Bottoms over the 2-year period. Mosquito abundance reached a peak following heavy spring rains and the emergence of "floodwater" species such as *Aedes nigromaculis* and *A. dorsalis.* No species became abundant until June (Table II). *Psorophora ciliata* (Fabr.), *P. cyanescens* (Coq.), *Anopheles punctipennis* (Say), and *Orthopodomyia signifera* (Coq.) were captured only in small numbers and were not considered potential vectors. *Culex pipiens* L. was captured numerous times but never in large numbers and evidently was not plentiful enough to be an important vector. *Culiseta inornata* (Williston) was collected in relatively large numbers only during April and October and was probably not important as a vector.

Traps baited with starlings, located in fields bordering the marsh, captured only *Culex tarsalis* Coq., *Aedes nigromaculis* (Ludlow), and *A. sollicitans* (Walker), although these areas were also those in which the other *Aedes* were most numerous. In 12 trap nights, 115 mosquitoes were captured: 55 engorged *Culex tarsalis*, 29 engorged, and 14 nonengorged *Aedes nigromaculis*, and 14 engorged and 3 nonengorged *A. sollicitans.* The local biology of these potential vectors is briefly as follows:

Aedes nigromaculis: Adults were collected from 19 May to 25 October. Principal production areas were the flooded fields bordering the marsh, in the late spring, and adults were most numerous in these areas. Adult abundance reached a peak in early summer and declined steadily until October. Adults captured in late summer and early fall showed the effects of wear. This species viciously attacked persons entering the areas bordering the marsh and was captured in traps baited with starlings.

Aedes sollicitans: Adults were collected from 19 May to 25 October. The local biology of this species paralleled very closely that of *A. nigromaculis* although it was not as abundant as the latter. Both species fed any time during the day or evening.

Culex tarsalis: Adults and larvae were collected as early as 7 May, larvae were taken in large numbers in September, and adults were collected until 25 October. Principal breeding places were roadside ditches, but adults could be captured at most locations within the Cheyenne Bottoms, including the fields bordering the marsh. This species was the most abundant throughout the mosquito season and was the only one captured in large numbers during the hot, dry period from mid-July to late August. Adults were readily taken in starling-baited traps even in those areas where they were not observed to bite man.

Efforts were made to infect the common local species of mosquitoes with *Plasmodium hexamerium* found in the starling. Attempts to collect and rear to adulthood adequate numbers of *Aedes nigromaculis, A. sollicitans,*

TABLE III. *Attempts to infect mosquitoes with* P. hexamerium.

	Exposure nights	No. surviving 10 days	Oocysts	Sporozoites
Culex tarsalis	70	all (45)	yes (10 days)	yes (18 days)
Culex pipiens	20	none	no	no
Culiseta inornata	80	2	no	no
Aedes dorsalis	30	none	no	no

and *A. vexans* larvae for feeding experiments were not successful. Under local field and laboratory conditions, approximately 200 to 300 third- or fourth-stage larvae were found to be required from the field to insure 15 to 20 newly emerged females in the laboratory. Attempts to infect the remaining species are summarized in Table III. "Exposure nights" denotes number of mosquitoes used in an attempt, multiplied by the number of nights an infected bird was placed in the cage. Of the species tested under laboratory conditions, only *Culex tarsalis* fed readily on the birds and became infected with *Plasmodium hexamerium.*

DISCUSSION

Vectors

Functional vectors of plasmodia are generally those mosquitoes which are abundant, feed readily and often on the vertebrate host, and are susceptible to the parasite (Bates, 1949; Russell, 1959). In this study, consideration of these requirements allows elimination of several species of local mosquitoes as possible vectors of *Plasmodium hexamerium* in the meadowlark and starling.

Anopheles punctipennis, Psorophora ciliata, P. cyanescens, Orthopodomyia signifera, Culiseta inornata, and *Culex pipiens* were shown to be of minor importance, numerically, in the Cheyenne Bottoms, as compared to other species. Of the above species only *Culex pipiens* has been shown capable of regularly transmitting avian plasmodia (Huff, 1965), but my results are similar to other studies (Reeves et al., 1954) in which the closely related *C. quinquefasciatus* was not considered numerous enough to serve as an important vector.

Of the remaining species studied, *Culex tarsalis* must be considered the most likely vector because of its abundance and ornithophilic feeding habits (Horsfall, 1955; Reeves et al., 1963; Tempelis et al., 1965). This species is considered a potential vector of avian malaria and encephalitis viruses wherever it occurs in large numbers. Rosen and Reeves (1954) have implicated it in California, and it is locally the most abundant and widely distributed mosquito of the Cheyenne Bottoms.

All of the "floodwater" *Aedes* collected occur in large numbers throughout the meadowlark nesting areas but the baited trap collections indicate that only *A. nigromaculis* and *A. sollicitans* feed regularly on small birds and therefore warrant consideration as local vectors.

The malaria incidence in nesting birds is high (Hewitt, 1940). The vector(s) for *P. hexamerium* must therefore have a close ecological association with the birds. *Culex tarsalis, Aedes nigromaculis,* and *A. sollicitans* have such an association. Infectivity experiments indicate *C. tarsalis* meets the requirements for a functional vector but do not eliminate the two *Aedes* species from consideration. *A. sollicitans* was shown to be susceptible to *Plasmodium cathemerium* (Herman, 1938), but this fact sheds little light on the local problem. With respect to the definitive host, probably *C. tarsalis* and possibly *A. nigromaculis* and *A. sollicitans* are responsible for transmitting infections under local natural conditions.

Total mosquito population does not reach a high density until June and it seems unlikely that much annual transmission occurs before that time.

Vertebrate hosts

The incidence of *Plasmodium hexamerium* in the meadowlark shows marked seasonal fluctuations (Fig. 1) with spring peaks that occur before the mosquito season. The weighted mean dates for the 1963 and 1964 spring meadowlark collections, using 1 March as the origin, are 5 April and 10 April, respectively. If one assumes that the spring peak is due to new infections, then the vector(s) must have been active in sufficient numbers

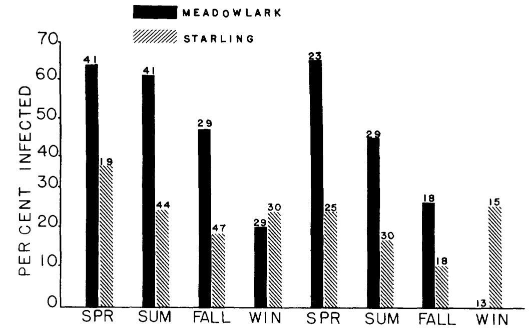

FIGURE I

FIGURE 1. Seasonal incidence of *Plasmodium hexamerium* in the meadowlark and starling. Numbers above the bars indicate the number of birds in the seasonal samples.

to create a threefold increase in malaria incidence at least 20 days prior to 5 to 10 April. (Twenty days is the approximate minimum time for sporozoite development plus completion of the preerythrocytic cycle in the bird.) My collection data indicate that this situation was not true for any mosquito species, particularly the most likely vectors.

With new infections ruled out as the cause, early spring incidence peaks in the meadowlark are probably best explained by meadowlark migration and/or relapse of the infection. Similar spring malaria incidence peaks in other birds have likewise been attributed to these factors (Manwell, 1955), both of which probably contribute to the observed incidence pattern: Lanyon (1957) showed that following migration, the meadowlark returns year after year to a very restricted nesting area; Janovy (1964) observed a much lower infection rate with an identical or very similar parasite in meadowlarks from areas of the Great Plains other than the Cheyenne Bottoms; and the works of Chernin (1952) and Dorney and Todd (1960) demonstrate that a correlation between seasonal factors, such as reproductive activity, and relapse in hemosporidian infections can exist.

From the above discussion, perhaps the best description of the epidemiological pattern, with respect to the meadowlark, is:

(1) There is a high endemic infection rate with *P. hexamerium* in locally breeding meadowlarks.

(2) The infection rate in locally collected meadowlarks drops in winter due to southern migration of the nesting population.

(3) The infection rate rises again in the spring with the replacement of the winter population by the nesting population.

(4) The young of the year become infected at a very early age, even in the nest.

(5) Relapse or other characteristic of the infection (e.g., a very long patent period) are probably what insure that circulating parasites will be available to the vectors when they become numerous enough to effect transmission later in the spring.

The seasonal incidence of *P. hexamerium* in the starling does not fluctuate significantly (Fig. 1). This situation is possibly a result of several factors, including a long patent period, low virulence of the infection, and lack of strong migratory tendencies in a large portion of the starling population (Kessel, 1953). A long patent period would insure that birds acquiring a primary infection in summer or early fall would circulate parasites into or through the winter. Thus the summer infection rate would tend to be maintained. The low virulence would tend to reduce the frequency and magnitude of epizootics, thus reducing incidence variation. Finally, lack of strong migratory tendencies would reduce large-scale movements of infected birds, thus reducing variation in incidence as measured by blood smear surveys.

Age incidence data indicate that starlings tend to become infected early in life, but not in the nest. The birds' local nesting ecology probably accounts for this situation. Nests are located deep in holes high in trees and much of the nesting occurs in the spring when winds are strong. This combination of factors would tend to isolate at least the early broods of starlings from the vectors, thus the birds would be exposed only when they begin to forage with the adults. It should be noted, however, that starling nestlings were collected in May, and perhaps later broods, hatched at the height of the mosquito season, do become infected in the nest.

Therefore, a possible description of the epidemiology of *P. hexamerium* in the starling, is:

(1) There is a fairly high endemic infection rate.

(2) The infection rate in locally collected starlings remains relatively constant during the

year due to the nonmigratory habits of a large portion of the starling population, a long patent period of the infection, and relatively low virulence of the parasite.

(3) Young of the year, at least the early broods, become infected after they leave the nest and their ecology approaches that of the adult birds.

P. hexamerium incidence in both meadowlark and starling is quite high for avian malaria (Hewitt, 1940). This observation, along with those that the starling's ecology matches that of the meadowlark most closely during the day, again indicate that the day-biting *Aedes nigromaculis* and *A. sollicitans* must be considered potential vectors.

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