205

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APPROVED BY

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

EPIDEMIOLOGY OF MALARIA IN CERTAIN BIRDS OF THE CHEYENNE BOTTOMS, BARTON COUNTY, KANSAS

CHAPTER I

INTRODUCTION

The vast medical importance of human malarias has led to their epidemiology becoming one of the best known and most extensively studied aspects of the diseases. On the other hand, the natural transmission of avian malaria remains one of the least understood aspects of the biology of the plasmodia. This lack of understanding was a prime motivation for this study.

In the past, the greatest obstacles to the study of avian malaria epidemiology have been ornithological problems. Herman's (1938) classical study of the natural transmission of malaria in the Redwing (*Agelaius phoeniceus*) is an outstanding example of how ornithological considerations can dictate the host-parasite systems studied. Herman's choice of the Redwing was greatly influenced by his ability to trap these birds, the availability of adequate numbers of nestlings, etc.

Practical considerations also influenced my choice of the specific birds and parasites studied, but this selection was based primarily on ecologic characteristics of the birds. The six species chosen for study were: American Coot (*Fulica americana*), Lesser Yellowlegs (*Totanus flavipes*), Long-billed Dowitcher (*Limnodromus scolopaceus*), Starling (Sturnus vulgaris), Redwing (Agelaius phoeniceus), and Meadowlarks (Sturnella magna and S. neglecta). The same birds were selected by Dr. J. T. Self and Dr. L. V. Scott for studies of the seasonal variations in the helminth parasite and arbovirus infections of these birds in the Cheyenne Bottoms Waterfowl Management Area, Barton County, Kansas.[1]

The guiding philosophy of this study was that of Boyd (1949), viz.,

"Any attempt to delineate the epidemiological picture presented by malaria must sketch the influence of:

1. The factors based on the relationship of the parasites to their intermediate hosts;

2. The extent to which ecologic characteristics of the intermediate host help or hinder transmission;

3. The factors based on the relationship of the parasites to their definitive hosts and;

4. The extent to which ecologic characteristics of the definitive host help or hinder transmission."

The "intermediate host" in the above statement refers, of course, to the vertebrate, and "definitive host" refers to the mosquito. Boyd's philosophy, which applies to avian as well as the human malarias for which it was established, implies that epidemiological patterns tend to be species specific with respect to both the parasite and the host. This basic principle, however, has yet to be demonstrated for avian malaria. It is this principle which I have attempted to demonstrate in the present study, particularly with respect to Plasmodium hexamerium in the Starling and Meadowlark. Application of Boyd's philosophy also

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1. This work was done while participating as a research assistant in this project supported by NIH Grant AI 05232-02 which also provided many of the facilities utilized.

requires the use of information from parasitological, ornithological, and entomological sources. This procedure was followed in the present study to gain information which adds to the epidemiological knowledge of malaria in various species of birds of the Cheyenne Bottoms.

The Study Area

The Cheyenne Bottoms Waterfowl Management Area is a prairie marsh located between Hoisington and Great Bend, Barton County, Kansas (Fig. 1). It is a land depression covering almost 30 square miles. The water level in the marsh is controlled by a system of dikes, gates, and canals and is maintained at a depth of about two to three feet except when certain pools are drained or allowed to dry for management purposes. An inlet canal carries water from Walnut Creek and a drainage canal empties into Cow Creek. Cattails, sawgrass, sedge, and millet (planted) are the principal marsh grasses and dense cattail covers much of the permanent water area. The outlying edges of Pools 2, 3, 4, and 5 end indefinitely in pastures and cultivated fields and extensive shallow flooding of these areas occurs following heavy rains. There are several stands of tall cottonwoods within two to three miles of the water's edge.

Rainfall is usually heaviest during the late spring and early fall. Spring is characterized by almost continuous strong, southerly winds, blowing dust, and violent thunderstorms including tornadoes. Summer is typically dry with extended periods of daytime temperatures of over 100° F. In winter there may be blowing sleet and snow with temperatures of lower than 10° F for extended periods of time.

The area is a major migration resting center and nesting ground for waterfowl, shorebirds, herons, coots, and blackbirds. The relative accessibility of large numbers of many species of birds makes the area a particularly favorable one on which to conduct bird parasite and arbovirus studies.

The wildlife management, public hunting, and refuge activities of the area are under the control of the Kansas Forestry, Fish, and Game Commission and are directed by Mr. Marvin D. Schwilling.

Review of the Literature

Many studies have dealt with the incidence of protozoan blood parasites in birds. The results of these studies have been summarized and discussed by, among others, Hewitt (1940), Levine (1962), Mackerras and Mackerras (1960), and Micks (1949). Generally, these surveys show that many species and/or strains of *Plasmodium*, *Leucocytozoon*, and *Haemoproteus* parasitize birds, that some enjoy a wide geographical and host range, and that incidence of infection varies with respect to the species of bird, the season, and the geographical location (Coatney and Roudabush, 1937; Dorney and Todd, 1960; Herman *et al*, 1954; Mackerras and Mackerras, 1960; Manwell, 1955; Micks, 1949). These works are in a sense epidemiological, but they tend to show only the vertebrate aspect of the entire parasitic cycle.

A few studies have dealt in detail with the transmission of avian plasmodia under natural conditions (Herman, 1938; Herman *et al*, 1954). Herman's (1938) study of the natural transmission of malaria in the Redwing stands as a classic work in this field and remains a standard by which all further works will be judged. Herman showed that the birds generally became infected after leaving the nest rather than as nestlings, and he was not only the first fully to recognize and try to understand the influence of bird ecology and behavior on the transmission of plasmodia, but he was also the first to conduct concurrent mosquito studies in an attempt to pinpoint the vectors. Later, Herman et al (1954) conducted similar studies in California and showed seasonal, geographical, and age differences in plasmodial infections.

Any discussion of the epidemiology of bird malaria or malaria-like organisms must include the definitive works by Fallis and his co-workers on the biology of Leucocytozoon in ducks (Fallis, Davies, and Vickers, 1951; Fallis, Anderson, and Bennett, 1954; Fallis, Pearson, and Bennett, 1954; Fallis and Bennett, 1963). Over a period of several years, Fallis and his colleagues were able to implicate certain simuliids as vectors, to delimit the dates of maximum transmission, and to show how certain ecologic characteristics of both the flies and ducks affect transmission.

Apparently there is little proof to date that in any given locality a single species of mosquito actually functions as the principal vector for a species of avian Plasmodium. Laboratory studies have, however, shown many species of mosquitoes to be susceptible to avian plasmodia and certain field studies have shown some of these to have a high vector potential (Reeves et al, 1954; Rosen and Reeves, 1954). Hewitt (1940) summarized the attempts to infect several species of mosquitoes, gave a list of those found capable of transmitting the infection, and discussed the experimental epidemiology studies of Huff (1927, 1930) and Russell (1931). Huff (1930) was able to show that after feeding once, only about one fourth of Culex pipiens survived the sporozoite development period and fed a second time. Russell's work (1931), involving Plasmodium cathemerium in Culex pipiens, Culex quinquefasciatus, and Canaries, showed that transmission did not regularly occur unless a high percentage of the birds had circulating gametocytes. Vargas (in Boyd, 1949) gave a more recent summary of the knowledge concerning vectors. Most of the work which he cited concerns experiments designed to test the influence of various factors on a mosquito's susceptibility to infection by various species of Plasmodium. In general, strain of mosquito and strain of parasite have been shown to be very important in this respect. Huff's extensive experiments with Culex pipiens as the vector of Plasmodium cathemerium and P. relictum indicated that a multiplicity of factors, such as temperature, size of blood meal, gametocyte level in the bird, etc., determine the chances of a mosquito becoming infected even after feeding on an infected bird (Huff, 1927, 1929, 1930, 1931). Application of Huff's results to epidemiological problems requires that, for a given locality, a mosquito species must be shown to be susceptible to a certain species of Plasmodium before that mosquito species can be considered a likely vector. Vargas (1949) hinted at this requirement.

In recent years, the study of human malaria epidemiology has shown it to be a much more complicated problem than formerly believed (Russell, 1959). The work of MacDonald (1957), culminating in his mathematical expression of the factors responsible for the transmission of the disease, revealed the nature of much of this complexity. MacDonald's concepts are quite applicable to avian as well as human malaria, and their application to avian malaria as we know it at present emphasizes that avian malaria epidemiology is still very poorly understood.

CHAPTER II

METHODS

The majority of all species of birds examined were collected by shooting. Some Starlings were captured in barns by hand and nestlings were taken directly from nests. The latter were kept alive for ten 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. Blood smears from the heart, lung, and other tissues of shot birds were made in the field as quickly as possible after collection. Spleen measurements were made on Starlings and Meadowlarks at the time of autopsy for helminths, usually several weeks later. The birds were kept frozen during the intervening time.

Blood and tissue smears were stained in Giemsa and examined for at least ten minutes under oil immersion at 1000x. Parasitemia was estimated by the methods of Gingrich, Hartmann, and others as given by Hewitt (1940). At least 2000 erythrocytes were counted on every slide on which an estimate of parasitemia was made.

Estimation of spleen size was made by multiplying the length by the greatest diameter of the spleen. The averages of these products for infected and non-infected birds were used as the X's in the "t"-test for the difference between two means.

Seasons were arbitrarily established for the Coot, Redwing, Meadowlark, and Starling as follows: Summer: June - August; Fall;

September - November; Winter: December - February; Spring: March - May. Collections of Long-billed Dowitcher and Lesser Yellowlegs were separated simply as to "Spring" or "Fall" migrants. Seasonal incidence was calculated on the basis of these criteria.

Parasites were identified by use of the keys and taxonomic characters of Hewitt (1940), Huff (1935), and Manwell (1935). Identifications of parasites from Starlings and Meadowlarks were verified by Dr. Reginald D. Manwell, Syracuse University.

Mosquito collections were made from March through October of each year. Regular collections were made in many parts of the area throughout this time. Adults were captured by use of New Jersey light traps, both battery and 110V operated, dry ice and baited traps, sweep nets, and aspirators. Dry ice and bait traps were made from five-gallon alcohol cans. Both ends of these cans were removed, an inward-pointing screen cone was placed in one end, and a cloth sleeve was placed over the other. Adults were kept frozen in small jars until they could be identified, sometimes several weeks later. At the time of identification, males were placed in 70 per cent ethanol. These males were later bleached in hot 10 per cent KOH and their terminalia clipped off, returned to 70 per cent ethanol, and mounted in Hoyer's Medium for identification. Adult mosquitoes were identified with the keys of Carpenter and LaCasse (1955).

Larvae for identification purposes and infectivity experiments were collected by dipping at many locations. Larvae for rearing were placed in gallon jars in the field, and kept in these in the laboratory until pupation. Pupae were transferred to water in five-inch porcelain cereal bowls containing a few pieces of cork. These bowls were then placed in 12" x 15" screen cages and covered with paper cones, open at the top, to prevent adults from getting back to the water. Adults were fed daily on sugar water on cotton except when attempts were made to feed them on birds. Temperature varied from 75° - 85° F in the laboratory and cages were kept over pans of water to insure adequate humidity.

Relative population densities for each species were estimated by a modification of the methods of Huffaker and Back (1943) and Russell and Rao (1941). The numbers of mosquitoes collected by all methods were calculated for each month, and the percentage of that total monthly collection contributed by each species was used as the relative population density for the species. Some of the mosquitoes used in calculating these relative population densities were those captured and used by Dr. L. V. Scott for virus studies. Collection activities from month to month were comparable so monthly tabulations of the total number captured were used as an estimate of overall mosquito abundance.

The laboratory strain of Plasmodium hexamerium used in the infectivity experiments was isolated from a Starling captured in January, 1964. This strain was maintained in wild-caught Starlings by sub-inoculation, 0.75 ml of blood drawn from the jugular vein into 0.25 ml of saline-citrate being used as the inoculum.

Mosquitoes were fed on the Starlings by placing the birds, breast feathers plucked, in a 1/4 inch mesh hardware cloth tube. This tube, which held the bird immobile, was placed in the mosquito cage, with the bird on its back, overnight. Sugar water was removed from the cage 24 hours before the feeding attempts.

Mosquitoes which were allowed to feed on infected birds were dissected for oocysts after ten days and for sporozoites at varying intervals thereafter. Stomachs and salivary glands were removed, preserved, and mounted according to the methods of Hunter *et al* (1946).

CHAPTER III

RESULTS

Vertebrate Hosts

Seasonal malaria incidence. None of 51 fall and 25 spring Lesser Yellowlegs or the 49 fall and 25 spring Long-billed Dowitchers examined were infected with blood parasites. Seasonal incidences of plasmodial infections for the Starling, Meadowlark, Redwing, and Coot are shown in Figures 2 and 3. Numbers of birds in the seasonal samples are shown above the bars giving the percentages of infection for the respective seasons.

Incidences for the Starling and Meadowlark are for *Plasmodium hexamerium* Huff 1930.

Parasitemia in the Coot and Redwing was less than one parasite per 10,000 erythrocytes. This exceedingly low parasite level and the scarcity of asexual stages in the circulating blood made identification to species impossible. Both plasmodia, however, had elongate gametocytes.

One Coot and one Starling were infected with species of *Plasmodium* other than those found regularly. The Coot (JJ2171) had a severe infection with a *Plasmodium* species which I have identified as *P. lophurae* Coggeshall 1938 (Fig. 8) and the Starling (JJ2513) had a dual infection with *Plasmodium hexamerium* and *P. relictum* (Grassi and Felletti) 1891.

Contingency table analyses (Dixon and Massey, 1957) of the data shown in Figures 2 and 3 indicate that incidence of infection in the Starling and Coot is independent of season at the five per cent level of significance (p/independence/ between 0.05 and 0.10 for the Starling and between 0.20 and 0.30 for the Coot), and that infection incidence in the Redwing and Meadowlark is <u>not</u> independent of season (p/independence/ less than 0.005 for both birds) at the same level of significance.

Incidences for the Starling and Meadowlark are based on infections by the same species of Parasite (<u>P. hexamerium</u>). Certain aspects of the infections by this parasite were studied in further detail.

<u>Age at which infection is acquired</u>. Age incidence data are shown in Figure 6. These data do not involve enough birds to warrant statistical analysis. However, of three Meadowlarks taken from the nest on June 19, 1964, at the approximate age of three to four days, one showed a heavy infection with <u>P. hexamerium</u> after ten days in the laboratory. The spleen smear of this bird showed numerous doubly infected cells and a very high parasitemia. Of five Meadowlarks taken from the nest on July 1, ten days after hatching, none showed parasites in either the peripheral blood or spleen smear.

Of 25 Starlings taken from the nest on May 5, 1964, at the age of three to four days, 18 survived ten days in the laboratory. None of these birds showed circulating parasites on any of three smears taken two days apart. Twelve of these birds subsequently died and showed no infection in either the heart or spleen smears.

In later collections, four out of ten juvenal and young-of-the-year Meadowlarks were infected with <u>P. hexamerium</u>, and three out of twelve young-of-the-year Starlings were infected with the same parasite.

Morphological differences in the *P. hexamerium* strains from different hosts. Figures 7 and 8 show representative stages of *P. hexamerium* in the Meadowlark and Starling. The number of nuclei in apparently mature circulating segmenters tends to be higher in the Starling than in the Meadowlark (Figs. 6b, 7b). The gametocytes of the Starling strain tend to be larger and more elongate than those of the Meadowlark strain (Figs. 6c, d; 7c, d). Neither strain appears to be heavily pigmented, particularly in the asexual stages where one or two pigment granules is typical (Figs. 6b, 7b). The undifferentiated trophozoites and immature gametocytes of the Starling strain tend to lie close to the side of the nucleus of the host cell (Fig. 7a), while those of the Meadowlark strain tend to lie free toward the end of the cell (Fig. 6a).

Parasitemia and synchrony. Parasitemia in the Meadowlark is generally higher than in the Starling, ranging from 16 to 40 (average 28) parasites per 10,000 erythrocytes in the former and from fewer than one to 60 (average 15) parasites per 10,000 erythrocytes in the latter. The gametocyte counts, however, are approximately the same, being 10 - 20 per 10,000 erythrocytes in the Meadowlark and 10 - 30 per 10,000 erythrocytes in the Starling.

Figure 5 shows the percentages of the various stages of the life cycle appearing in the circulating blood of ten Meadowlarks and ten Starlings collected in the morning and ten Meadowlarks and ten Starlings collected in the evening. This graph implies that there is no marked synchrony in segmentation (as defined by Wolfson, (1936) in either bird; however, a tendency toward evening segmenter maturation can be seen in the Meadowlark. Periodicity, or length of the asexual cycle, was not determined for either strain.

Pathology, virulence, and the patent period. Spleen size of infected Meadowlarks averages significantly larger than that of non-infected birds at the five per cent level. Spleen size as calculated averaged 177 sq. mm. for 38 infected birds (maximum 10 x 50 mm) and 111 sq. mm. for 44 non-infected birds (maximum 8 x 30 mm). There was no significant difference between the spleen size of 28 infected and 30 non-infected Starlings. Spleens of infected Starlings averaged 91 sq. mm. while those of non-infected birds averaged 93 sq. mm. Maximum Starling spleen size was 7 x 30 mm. in a non-infected bird.

The course of laboratory infections transmitted by sub-inoculation conforms most closely with that of avian plasmodia of low virulence as summarized by Hewitt (1940). Circulating parasites did not appear until 15 to 29 days after sub-inoculation in four laboratory reared birds. The Starling strain of parasite also proved non-infective for nine English Sparrows (Passer domesticus) and three three-day-old Pekin ducks. Parasites could be demonstrated in the circulating blood of two of the four above laboratory-infected Starlings up to five months after the onset of the patent period. While accurate counts of the parasitemias resulting from these infections were not made, they were not obviously greater than those observed for natural infections. The Meadowlark strain was not maintained in the laboratory.

Ornithological Observations

Among the birds studied, such ecologic and behavioral characteristics as might influence malaria incidence were observed.

Coots apparently show very little migratory or other large scale movements during the mosquito season. Locally the birds nest principally in Pool 2. Before June and after July they can be seen in fairly large numbers throughout the permanent water area and along the edges of the dikes.

Both the Long-billed Dowitcher and Lesser Yellowlegs occur in large numbers along the edges of the pools in spring and fall.

Starlings remain principally in areas more than a mile from the water's edge, frequenting fields and feed lots during the day and roosting high in tall cottonwoods or barns, depending on the season, at night. Important nesting localities are cottonwood stands in section 34, T18S, R13W, and section 2, T19S, R13W. Nesting begins in late April and occurs typically in tree holes up to 60 feet above the ground.

Redwings nest in large numbers throughout most of the cattail areas. They spend much of their time in the cattails of the permanent water areas during the nesting and mosquito season, moving into large flocks and out into the adjacent fields and along the dike roads after the nesting season.

Meadowlarks occur in fairly large numbers throughout all of the area bordering the marsh and along the dike roads. Important nesting areas are south of Pool 5 in sections 35 and 36, T18S, R13W, sections 31 - 34 of T18S, R12W, and sections, 2, 3, 4, 9, 10, and 11, T18S, R12W north of Pool 3.

Vectors

Eleven species of mosquitoes were identified from the Cheyenne

Bottoms over the two year period. Overall mosquito abundance evidently reaches a peak in June following heavy spring rains and the emergence of "floodwater" species (Figs. 11, 13, and 14). Adult collection data indicate that none of the mosquito species become abundant until June (Fig. 11) and mosquitoes are not important as pests before that time. Four species, Psorophora ciliata (Fabricius) 1794, P. cyanescens (Coquillett) 1902, Anopheles punctipennis (Say) 1823, and Orthopodomyia signifera (Coquillett) 1896, were captured only a few times in small numbers and were not therefore considered numerous enough to warrant consideration as potential vectors of bird malaria (Fig. 10). Culex pipiens Linnaeus 1758, was captured many times but never in large numbers (Figs. 10, 12), and this species is evidently not plentiful enough to be considered important as a vector. Culiseta inornata (Williston) 1893, likewise, was collected many times but in relatively large numbers only during restricted times of the mosquito season (Fig. 12) and so is probably not important as a vector. The biology of the principal species of Cheyenne Bottoms mosquitoes is discussed below.

Culiseta inornata (Williston) 1893: Egg rafts and larvae were collected as early as April 14 and adults as late as October 25. Adults and larvae were exceedingly rare between June 1 and October 1 but relatively abundant during April and October (Fig. 12). Larvae were collected at several locations (ditches in section 27, T18S, R13W; section 11, T18S, R12W; and Section 2, T19S, R13W) but the sheltered drainage ditch on the Rusco farm in section 2, T19S, R13W, appeared to be a major breeding place. This species probably winters locally as an adult because all of the locations in which larvae were collected were dry throughout the winter.

Aedes dorsalis (Meigen) 1830: Larvae were collected as early as April 14, adults as late as October 25. This species was one of the most abundant throughout much of the mosquito season (Fig. 14). Local breeding places included most of the ditches along roads leading into the marsh. Locally, eggs of this species apparently hatch quickly after being emersed any time during the mosquito season, since larvae were collected in temporary pools during late summer when this was not possible for other "floodwater" species of *Aedes*.

Aedes nigromaculis (Ludlow) 1907: Adults were collected from May 19 to October 25. Principal production areas were the flooded fields bordering the marsh, in the late spring, and adults were most numerous in these areas (sections 35, 36, T19S, R13W; sections 31-34, T19S, R12W; sections 2-4, 9-11, T18S, R12W). Adults reached a peak abundance in early summer and decreased steadily in number until October (Fig. 13). This species attacks viciously persons entering the areas bordering the marsh. It was captured in traps baited with small birds.

Aedes sollicitans (Walker) 1856: Adults were collected as early as May 19 and as late as October 25. The local biology of this species paralleled very closely that of *A. nigromaculis* although it was not as abundant as the latter (Figs. 10, 13). *A sollicitans* readily bites persons entering the areas bordering the marsh any time of day or evening and was captured in traps baited with small birds.

Aedes vexans (Meigen) 1830: Adults were collected from May 4 (single specimen) to October 25. No larvae of this species were collected over the entire two-year period. I was unable to collect larvae in local roadside ditches, even those subject to intermittent flooding. The adult population appeared to be more stable than that of other "flood-water" Aedes (Fig. 14). A. vexans readily attacked man any time of the day or evening in the areas bordering the marsh, but was not captured in traps baited with small birds.

Culex pipiens Linnaeus 1758: Adults were collected in small numbers from May 19 to October 17. Principal collection sites were in the southern parts of section 27, T18S, R13W, and section 4, T19S, R13W. Larvae were collected in roadside ditches in these same areas in June. This species is evidently not a major one numerically (Figs. 10, 12).

Culex tarsalis Coquillett 1896: Adults and larvae were collected as early as May 7; larvae were taken in large numbers in September and adults as late as October 25. Principal breeding places were road-side ditches, but adults could be captured at most locations through-out the area, including the field bordering the marsh. This species was the most abundant throughout the mosquito season (Figs. 10, 12). It is the only species which was captured in large numbers during the hot, dry period from mid-July to late August. Adults were taken readily in traps baited with small birds, even in areas where they were not observed to bite men.

I was unable to collect mosquito larvae in the permanent waters of the marsh over the entire two-year period. Evidently the permanent water does not contribute significantly to the production of the total mosquito population. The grassy areas along the dikes, however, abounded with adults, particularly Aedes nigromaculis and A. sollicitans, which must have moved there from other areas.

Local feeding habits of the various species were not studied in detail. However, *Aedes dorsalis*, *A. nigromaculis*, *A. sollicitans*, *A. vexans*, *Psorophora ciliata*, and *Culex tarsalis* were all observed to bite or attempt to bite man under various field conditions. Engorged females were not captured in sufficient numbers to lend statistical meaning to precipitin tests, although anti-passeriform serum, prepared according to the method of Templis and Reeves (1962) was available. Traps in areas north of Pool 3 and south of Pool 5 baited with Starlings captured only *Culex tarsalis*, *Aedes nigromaculis*, and *A. sollicitans*. These traps did not capture large numbers of individuals (Fig. 9), but the proportions of the three species in the baited-trap collections indicate that all three will readily feed on small birds near the ground. Most individuals captured in the baited traps were engorged (Fig. 9). Laboratory experiments (see below) indicate that local strains of *Psorophora ciliata*, *Aedes dorsalis*, and *Culex pipiens* will also feed on small birds under certain conditions.

Infectivity experiments. No attempt was made to infect *Anopheles punctipennis*, *Orthopodomyia signifera*, or *Psorophora cyanescens* with the laboratory strain of *Plasmodium hexamerium*. Attempts to collect and rear to adulthood adequate numbers of *Aedes nigromaculis*, *A. sollicitans*, and *A. vexans* larvae for feeding experiments were not successful. Under laboratory and field conditions, approximately 200 - 300 third or fourth stage larvae were found to be required from the field to insure 15 - 20 newly emerged females in the laboratory. Attempts to infect the remaining species of mosquitoes are discussed below.

Culiseta inornata: An infected Starling was exposed to 15 - 20 newly emerged females on May 10, 12, 14, and 16, 1964, and none appeared to engorge. Only two of these mosquitoes survived ten days and neither showed oocysts or sporozoites.

Psorophora ciliata: A single newly emerged female fed partially on an infected Starling on September 5, 1964. It died on September 10 and no oocysts were observed on the stomach.

Aedes dorsalis: An infected Starling was exposed to 15 females on May 10 and 12, 1964. None fully engorged and all died within ten days. None showed oocysts or sporozoites upon dissection although one was partially engorged.

Culex pipiens: An infected Starling was exposed to 10 newly emerged females on May 23, 1964. Most of these appeared to have fed partially. All but two died during the ten-day incubation period and these two, dissected on June 4, contained no oocysts. An infected Starling was exposed to 8 - 10 newly emerged females on June 2. Apparently none fed and all died during the ten-day incubation period.

Culex tarsalis: An infected Starling was exposed to 15 - 20 newly emerged females on May 23, 1964, and all engorged. Eleven small oocysts were found in one mosquito on May 31 and up to 30 fully developed oocysts were found in several others on June 4. About 25 newly emerged females were given an infected Starling on September 5 and 6, 1964, and all engorged. Three dissected on September 22 contained both oocysts and sporozoites.

Of the species tested under my laboratory conditions, only *Culex tarsalis* fed readily on the birds and became infected with the laboratory strain of *Plasmodium hexamerium* used.

CHAPTER IV

DISCUSSION

Diagnostic Methods

The accurate diagnosis of avian malaria infections has always been a problem in survey work. Examination of single thin smears of peripheral blood, while the poorest of diagnostic methods, has been the principal method used in many important surveys (Coatney and Roudabush, 1937; Coatney and West, 1938; Couch, 1952; Mackerras and Mackerras, 1960). Examination of heart, spleen, and lung smears generally reveals a higher malaria incidence, and is thus said to be a more accurate diagnostic method, than the examination of peripheral smears alone (Herman, 1938; Hewitt, 1940). The principal advantage to blood or tissue smear examination is that this method can easily be standardized and used consistently over a lengthy period and for all aspects of a survey. Xenodiagnosis, or sub-inoculation of blood from wild-caught birds into susceptible laboratory hosts, reveals the greatest number of infections and is thus said to be the best method of diagnosis (Hewitt, 1940). The principal disadvantages of this method are: (1) not all strains or species of *Plasmodium* are infective to laboratory hosts (Hewitt, 1940; Huff, 1963), (2) large colonies of parasite-free laboratory hosts are necessary but are often expensive and difficult to acquire, and (3) the often serious ornithological problem of not being able to trap at will large numbers of the desired bird species must be solved. These disadvantages have led to the xenodiagnostic method being used inconsistently in conjunction with other methods, and to the choice of hosts being those which one is able to trap easily (Herman, 1937; Herman _et al_, 1954).

The diagnostic method used in this study, while admittedly not the one generally considered the most accurate, was nevertheless used consistently on all species of birds at all times of the year. I feel that the gain in consistency offsets the loss of accuracy suffered by use of the heart-blood and lung-smear method.

Vectors

Functional vectors of plasmodia are generally considered to be those species of mosquitoes which are abundant, which feed readily and often on the vertebrate host, and which are highly susceptible to infection by the plasmodia (Bates, 1949; Herms and James, 1961; Russell, 1959). These requirements are as applicable to potential vectors of avian plasmodia as to the vectors of the human plasmodia for which they were established. In the present study, consideration of these requirements allows elimination of several species of local mosquitoes from the list of possible vectors of _Plasmodium hexamerium_ in the Meadowlark and Starling.

Anopheles punctipennis, _Psorophora ciliata_, _P. cyanescens_, _Culex pipiens_, and _Culiseta inornata_ were shown to be of minor importance numerically, in the Cheyenne Bottoms, as compared to other species (see "Results", and Fig. 10). Although quantitative data such as that required by MacDonald's concept of _critical vector density_ are not provided by this study, it is evident that the populations of the above species are not large enough to warrant consideration of the species as important vectors. Culex pipiens is the only one of the above species which has been shown to be capable of regularly transmitting avian plasmodia under laboratory conditions, but my results are similar to other field studies (Rosen and Reeves, 1954) in which the very closely related Culex quinquefasciatus Say 1823, was considered not numerous enough to serve as an important natural vector of avian malaria.

Of the remaining species studied, Culex tarsalis must be considered the most likely local vector because of its abundance (Figs. 10, 12) and ornithophilic feeding habits (Horsfall, 1955; Reeves et al, 1954; Reeves et al, 1963). This species is generally considered an important vector of avian malaria wherever it occurs in large numbers, and Rosen and Reeves (1954) have implicated it as such in California. Culex tarsalis can be collected in almost any area of the Cheyenne Bottoms and it occurs in great numbers throughout all of the principal Meadowlark nesting areas.

All of the "floodwater" Aedes collected (A. dorsalis, A. vexans, A. nigromaculis, and A. sollicitans) occur in relatively large numbers, at least in certain areas of the Cheyenne Bottoms (Figs. 10, 13, 14). They are most abundant along the dike roads and throughout the principal Meadowlark nesting areas. Although all four species are abundant, baited-trap collections in the fields bordering the marsh (Fig. 9) indicate that only A. nigromaculis and A. sollicitans feed regularly on small birds and therefore warrant consideration as potential vectors of local avian plasmodia.

Collection results (Figs. 9-14) indicate that total mosquito population, and therefore the population of any one species, does not reach large proportions until June. Although relative populations of some species, e.g. Culiseta inornata and Aedes dorsalis, are quite high during April and May, the actual number of mosquitoes captured during these months is low as compared to the rest of the mosquito season. It seems unlikely, therefore, that much annual transmission of avian malaria occurs before June.

Vertebrate Hosts

Redwing: Epidemiological data for the Redwing and Coot are neither extensive nor specific enough to permit many conclusions regarding the transmission of malaria in these birds. The Redwing graph (Fig. 3), however, has certain characteristics which indicate that the epidemiological picture may be similar to that observed by Herman (1938), who demonstrated that Redwings on Cape Cod, Massachusetts, do not acquire primary infections until after they leave the nest. My Redwing data show the highest incidence in summer in each of two consecutive years. The low parasitemia in these birds, however, casts doubt on the concept that this peak results from new infections; at least, one would expect a primary infection with any species of avian plasmodium to show a parasitemia of greater than 1/10,000 (Hewitt, 1940). However, this high incidence occurs not only just after large numbers of fledglings leave the nest, but also shortly after the peak in total mosquito population. Unfortunately, the proportions of immature birds in the summer samples are not known, so their contribution to the total incidence is not known. There is also no way of determining, from the data at hand, whether the summer increase is due largely to factors such as relapse (cf. Chernin, 1952), or the influx of Redwings from other areas with a higher malaria incidence. I believe that the summer peak in incidence of malaria in these birds is due largely to factors other than new infections in young of the year, principally because of the low parasitemia in all Redwings even though the intensity of primary infections in these birds is now known.

Coot: It is possible that the malaria incidence graph for the Coot (Fig. 3) represents infections by *Plasmodium lophurae*. However, the one bird in which the parasite could be identified had a severe infection, while the remainder of the infected Coots showed a parasitemia of less than 1/10,000 (see "Results"). The severe infection is therefore atypical and a low grade infection is the usual case. *Plasmodium lophurae* is generally regarded as a highly virulent parasite, and has, in fact, been shown to be quite pathogenic to the American Coot (Jordan, 1957). It seems unreasonable, therefore, to assume that all infected Coots carried the parasite which I have identified as *P. lophurae* unless the low grade infections were in the relapse stage or represent secondary infections in partially immune birds. Chernin (1952) has shown that a highly virulent haemosporidian, *Leucocytozoon simondi*, could produce low grade relapses in ducks, and Hewitt's (1940) discussion of immunity indicates that attempted superinfection with the same strain of parasite results in the rapid removal of parasites from the circulating blood. Therefore, either relapse and/or immunity could contribute to the observed parasitemia.

The one severe infection could, of course, represent a fortuitous one by a species of *Plasmodium* not "natural" to the Coot. Hewitt

(1940) has summarized the work concerning avian plasmodia in abnormal hosts. This summary indicates that certain avian plasmodia are infective to birds of little phylogenetic relationship and that the morphology and physiology of the parasites are changed in the abnormal host. Thus, this explanation of the observed parasitological situation should not be ignored.

Little published information is available concerning Plasmodium infections in the Coot. Roudabush (1942) reported the common passeriform parasite P. relictum from the American Coot in Nebraska, and Jordan (1957) demonstrated that P. lophurae was highly pathogenic to the same host. Neither of these records adds much to the epidemiological picture of malaria in this bird, and about all that can be derived from the present study is that apparently season-dependent factors do not influence greatly the incidence of Plasmodium infections in local Coots.

Meadowlark: The incidence graph for Plasmodium hexamerium in the Meadowlark shows marked seasonal fluctuations which tend to be consistent for a two-year period (Fig. 2). Perhaps the most significant fact concerning these fluctuations is that the spring peak occurs before the mosquito season. The weighted mean dates for the 1963 and 1964 spring Meadowlark collections, using March 1 as the origin (Hoel, 1954), are April 5 and April 10 respectively. If one assumes that the spring peak is due to new infections, then, considering the bionomics of the various species of local mosquitoes (see above discussion, also "Results", and Figs. 9-14), only Culiseta inornata and Culex tarsalis can even remotely be considered responsible for these new infections.

Culiseta inornata is generally considered an early spring and fall, or cool weather, mosquito in the temperate zones, and probably passes the winter locally as an adult (Carpenter and LaCasse, 1955; Horsfall, 1955). Both eggs and larvae of this species were first collected on April 14, indicating that adults were active at the time. The malaria incidence, however, reaches its peak five to ten days earlier. This means, theoretically, that the vector would have to occur in sufficient numbers to create this three-fold increase in malaria incidence at least 20 days prior to April 5 - 10. Twenty days is the approximate minimum time for sporozoite development plus completion of the exoerythrocytic cycle in the bird. The mosquito collection data (see above) indicate that this was not true for *Culiseta inornata*.

Likewise, *Culex tarsalis*, which is said to hibernate as an adult, and which is the most likely vector considering the laboratory experiments, bionomics (see above), and published information (Horsfall, 1955; Reeves *et al*, 1954; Rosen and Reeves, 1954), would have to be present and active in sufficient numbers to cause a three-fold increase in malaria incidence by mid-March. Again, my data indicate that this situation did not exist.

Therefore, with new infections ruled out as the cause of early spring incidence peaks in the Meadowlark, only relapse, migration, or a combination of the two can be considered responsible for the peaks. The data at hand do not reveal which of these explanations is most likely, but cases of varying strength can be built for all three.

The works of Chernin (1952) and Dorney and Todd (1960), demonstrated that a correlation between seasonal factors, such as reproductive activity, and relapse in haemosporidian infections can exist.

Attributing the spring incidence peak entirely to relapse, however, requires that the Meadowlark be strictly non-migratory. But Lanyon (1957) has shown the Meadowlark to be migratory, and in fact, migratory in such a way as to create sharp, repeated, seasonal fluctuations in their parasite fauna as determined by a random collection of birds from a particular area. He states that the Meadowlark returns year after year to a very restricted nesting area. The observed malaria incidence fluctuations could therefore result if the infection rate in locally breeding birds was about 65 per cent (Fig. 2), if these birds were replaced in winter by Meadowlarks from farther north in which the infection rate with *Plasmodium hexamerium* was much lower (viz., 21 per cent), and if the locally breeding birds returned to the Cheyenne Bottoms to nest year after year. The 65 per cent incidence in nesting birds implies a high rate of local transmission, and, that such a rate occurs is shown by the rapid acquisition of infections by young of the year (Fig. 4). In addition, Janovy (1964) has demonstrated a much lower infection rate with an identical or closely related parasite in Meadowlarks of another area of the Great Plains. Thus considerable credibility is lent to the explanation of the incidence variations based on migration.

Perhaps the most plausible explanation of the incidence fluctuations is based on a combination of Meadowlark migration *and* relapse or some other characteristic of the parasitic infection. Some birds collected in late winter and early spring had circulating parasites. Therefore, the infection must *either* relapse in late winter or early spring, *or* the birds must circulate parasites through the winter. Lanyon's work (1957) on Meadowlark migration (see above) cannot be ignored, thus, both the migratory habits of the host and the characteristics of the parasitic infection must contribute to the causes of the observed incidence fluctuations.

From the above discussion, the most logical set of circumstances describing the epidemiological pattern, with respect to the Meadowlark, is:

(1) there is a high endemic infection rate with *Plasmodium hexamerium* in locally breeding Meadowlarks;

(2) the infection rate in locally collected Meadowlarks drops in winter due to southern migration of the breeding birds and replacement of them by birds from farther north in which the malaria incidence is relatively low;

(3) the infection rate in locally collected birds rises again in the spring with the return to the Cheyenne Bottoms, of the nesting population;

(4) the young of the year become infected at a *very* early age, even in the nest; and

(5) relapse or other characteristics of the parasitic infection insure that circulating parasites will be available to the vectors when their population reaches the density required to effect new transmission later in the spring or summer.

The 65 per cent incidence in nesting birds is very high for avian malaria (Hewitt, 1940). The vector(s) for *P. hexamerium* must therefore remain in close ecological association with Meadowlarks, feed readily and often on the birds, and be highly susceptible to the parasite. *Culex tarsalis* and all of the "floodwater" *Aedes* collected have close ecological association with Meadowlarks, but only *Culex tarsalis*, *Aedes* nigromaculis, and A. sollicitans were shown to feed regularly on small birds under local natural conditions. All three species occur in great numbers throughout all of the principal Meadowlark nesting areas (see "Results"). The laboratory experiments described above indicate that Culex tarsalis meets the requirements for a functional vector, but they do not eliminate the two Aedes species from consideration. Aedes sollicitans has been 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, one can only say that probably Culex tarsalis and possibly Aedes nigromaculis and A. sollicitans are responsible for transmitting the infections under local natural conditions.

Starling: The seasonal incidence of Plasmodium hexamerium infections in the Starling does not fluctuate significantly (Fig. 2). This situation is probably a result of several factors, including the 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 primary infections in summer or early fall would circulate parasites into or through the winter. Thus the summer infection rate would tend to be maintained in the winter. The low virulence of the infection would tend to reduce the frequency and magnitude of epizootics, thus reducing seasonal incidence variation. And finally, lack of strong migratory tendencies would reduce large scale movements of infected birds, thus reducing variations in incidences as measured by blood-smear surveys.

The incidence is again quite high for avian malaria (Hewitt, 1940), but does not approach that in the nesting Meadowlarks. This relatively low incidence indicates either a lower susceptibility of the Starling to the parasite or an ecological association with the vector species which is not nearly as intimate as that of the Meadowlark with the same vector(s). Both situations probably contribute to the relatively low incidence. The ecology of the Starlings, particularly their roosts high in trees, tends to isolate these birds, more than the Meadowlarks, from the vectors during the critical evening hours. Splenic enlargement data (see "Results") and parasitemia counts indicate that the Starling is more resistant to the parasite than the Meadowlark.

Age incidence data (Fig. 4) indicate that Starlings tend to become infected early in life, probably shortly after fledging, but not while in the nest. The bird's local nesting habits probably account for this situation. The nests are located deep in holes high in trees, and much, if not most, of the nesting occurs in the spring when the winds are strong in the area. This combination of factors tends to isolate the nestlings from the mosquitoes, thus the young of the year are exposed to the vectors only when they begin to forage with the adults.

Therefore, a possible set of circumstances explaining the epidemiological pattern, with respect to the Starling, is:

(1) there is a fairly high endemic infection rate with *Plasmodium hexamerium* in local Starlings,

(2) the infection rate in locally collected Starlings remains relatively constant during the year due to the non-migratory habits of a large portion of the Starling population, a long patent period of the infection, and relatively low virulence

of the infection, and

(3) young of the year become infected only after they leave the nest and their ecology approaches that of the adult birds.

Again, 20 to 30 per cent is a high avian malaria incidence, and the parasite is the same species found in the Meadowlark. These observations, in combination with those that the Starling's ecology matches the Meadowlark's most closely during the day (see "Ornithological Observations"), indicate that the vector is a species which is active at least during the day. Thus, the day-biting *Aedes nigromaculis* and *A. sollicitans*, along with *Culex tarsalis*, must again be considered possible vectors.

The findings of this study with respect to *Plasmodium hexamerium* can now be related to Boyd's epidemiological philosophy as follows:

1. Factors based on the relationship of the parasites to their intermediate hosts: The relatively low virulence and long patent period of the infection in the Starling insures a circulating supply of parasites when the vector population reaches the level necessary to effect transmission. A similar patent period or a relapse phenomenon possibly occurs in the Meadowlark, thus providing the circulating supply of parasites from year to year.

2. The extent to which ecologic characteristics of the intermediate host help or hinder transmission: The ecologic characteristics of all ages of Meadowlarks brings them into intimate contact with the probable vectors at all times of the day and night, thus the young of the year tend to become infected at a very early age, even in the nest, and the endemic infection rate is very high for all ages. The migratory pattern of the Meadowlark helps to insure a return, every year, to the same

locality, of a large number of infected birds. The ecologic characteristics of the Starling, however, tend to protect the nestlings from infection and expose the adult birds to vectors only during the day. Thus young of the year tend to become infected only after they leave the nest and begin to forage with the adults. The migratory pattern of the Starling does not greatly influence the number of infected birds in the area.

3. Factors based on the relationship of the parasites to their definitive hosts: Not all of the mosquito species found in the Cheyenne Bottoms are known to become infected with *Plasmodium hexamerium*, but at least one of the most likely vectors, *Culex tarsalis*, does become infected.

4. The extent to which ecologic characteristics of the definitive host help or hinder transmission: The ecologic characteristics of eight of the eleven species of local mosquitoes tend to eliminate them as potential vectors. Characteristics such as population dynamics, feeding habits, and breeding biology leave only *Culex tarsalis*, *Aedes nigromaculis*, and *A. sollicitans* as probable or possible vectors for *Plasmodium hexamerium* in the Meadowlark and Starling.

I believe that the above results and ensuing discussion demonstrate that some of the implications of Boyd's philosophy are indeed true: in certain cases epidemiological patterns tend to be species specific with respect to the avian host. A similar analysis with another species of *Plasmodium* in the Meadowlark and Starling is, of course, required to demonstrate that epidemiological patterns are also species specific with respect to the parasite.

CHAPTER V

SUMMARY

A two year study of the epidemiology of avian malaria in certain birds was conducted in the Cheyenne Bottoms Waterfowl Management Area, Barton County, Kansas.

The American Coot (*Fulica americana*), Lesser Yellowlegs (*Totanus flavipes*), Long-billed Dowitcher (*Limnodromus scolopaceus*), Starling (*Sturnus vulgaris*), Redwing (*Agelaius phoeniceus*), and Meadowlark (*Sturnella* sp.) were surveyed for plasmodia by examining blood smears taken from the heart and lung.

Lesser Yellowlegs and Long-billed Dowitcher were found not to be infected with blood parasites. Incidence of *Plasmodium hexamerium* infections varied significantly with the season in the Meadowlark, with highest incidence in the spring (April) and lowest in the winter (December), but did not vary significantly in the Starling. The infection rate of *Plasmodium* sp. in the Redwing varied significantly, with highest incidence in the summer, but no significant variation was found for the infection rate with *Plasmodium* sp. (*P. lophurae*) in the Coot.

The epidemiology of *Plasmodium hexamerium* in the Meadowlark and Starling was studied in further detail to determine whether epidemiological patterns were specific for each host.

Age incidence data indicate that both the Meadowlark and Starling tend to become infected at a very early age. Meadowlarks may acquire a primary infection even while in the nest, but Starlings are not known to become infected until after they leave the nest. Differences in the nesting ecology of the two birds probably accounts for this epidemiological difference.

Concurrent mosquito studies contributed data concerning eleven species of potential vectors. The bionomics of eight of these mosquito species eliminates them from consideration as probable vectors of *Plasmodium hexamerium*. *Anopheles punctipennis*, *Psorophora ciliata*, *P. cyanescens*, *Orthopodomyia signifera*, *Culiseta inornata*, and *Culex pipiens* were not numerous enough to be considered important as vectors of *Plasmodium hexamerium*. *Aedes dorsalis* and *A. vexans* were not captured in traps baited with Starlings, even in areas in which these mosquitoes were most abundant, and consequently they are not considered important vectors. Baited-trap collections indicate that *Culex tarsalis*, *Aedes nigromaculis*, and *A. sollicitans* all feed readily on small birds under local natural conditions, and therefore warrant consideration as potential vectors.

Total mosquito collections indicata that (1) *Culex tarsalis* is the most abundant mosquito in the Cheyenne Bottoms, but *Aedes nigromaculis* and *A. sollicitans* also occur in very large numbers, and (2) overall mosquito population does not reach large proportions until June and annual transmission of avian malaria probably does not occur to any great extent before that time.

Laboratory experiments show that *Culex tarsalis* is also susceptible to the Starling strain of *Plasmodium hexamerium* and must be considered its most likely vector in the Cheyenne Bottoms. Larvae of *Aedes nigromaculis* and *A. sollicitans* were not collected in sufficient numbers to complete infectivity experiments.

The mosquito studies and Meadowlark incidence data indicate that spring malaria incidence peaks in the Meadowlark occur before vectors are numerous enough to effect transmission. Incidence fluctuations are believed to result from the migratory habits of the host and characteristics of the parasitic infection. Meadowlarks nesting in the Cheyenne Bottoms have an endemic infection rate of about 65 per cent. The incidence in locally collected Meadowlarks drops in winter, and this is believed to be due to southward migration of the locally nesting birds and replacement of them by birds from farther north with an infection rate of about 20 per cent. The infection rate in locally collected birds rises again to the endemic level with the return, in the spring, of the nesting population. Relapse and/or a very long patent period insure a supply of circulating parasites when the vectors become numerous enough to effect new transmission.

Meadowlark nesting occurs in those areas in which all three likely vectors are most abundant. This intimate ecological association with the vector(s) probably accounts for the high infection rate in locally nesting birds as well as the infections in their nestlings.

In the Starling, lack of strong migratory tendencies, relatively low virulence and a long patent period of the P. hexamerium tend to reduce respectively: (1) large scale movements of infected birds, (2) the frequency and magnitude of epizootics, and (3) a drop in observed infection rate in locally collected winter birds due to onset of latency. The lack of significant seasonal fluctuation is probably a result of all three factors. The ecological association of Starlings with Culex tarsalis, Aedes nigromaculis, and A. sollicitans is not as great as, and the incidence of P. hexamerium infections is much lower than, in the Meadowlark. The difference in degree of ecological intimacy probably accounts for the difference in infection rate and for the observation that Starling young of the year do not become infected until after they leave the nest.

The studies of Plasmodium hexamerium in the Meadowlark and Starling show that avian malaria epidemiology tends to be specific with respect to the species of vertebrate host.

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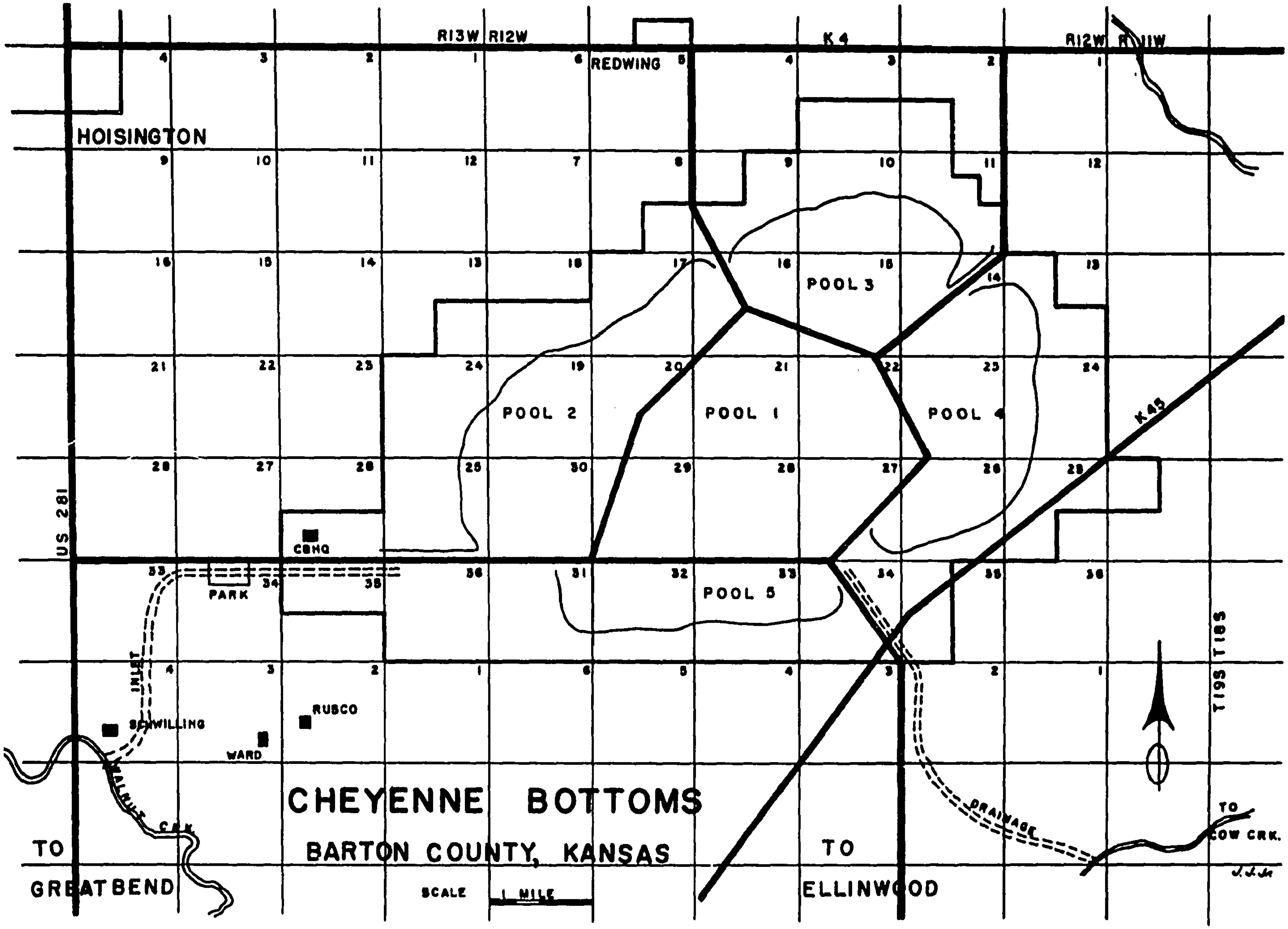
EXPLANATION OF FIGURES

Figure 1. Cheyenne Bottoms Waterfowl Management Area, Barton County, Kansas and Surrounding Area.


CHEYENNE BOTTOMS
BARTON COUNTY, KANSAS
R13W R12W
K 4
R12W R11W
REDWING
HOISINGTON
POOL 1
POOL 2
POOL 3
POOL 4
POOL 5
K 45
US 281
CBHQ
PARK
INLET
SCHWILLING
WARD
RUSCO
WALNUT CRK.
DRAINAGE
TO COW CRK.
T19S T18S
TO GREAT BEND
TO ELLINWOOD
SCALE 1 MILE

EXPLANATION OF FIGURES (continued)

Figure 2. Seasonal incidence (per cent infected) of *Plasmodium hexamerium* infections in Starlings and Meadowlarks.

Figure 3. Seasonal incidence (per cent infected) of *Plasmodium* sp. infections in Redwings and American Coots.

Figure 4. Seasonal incidence (per cent infected) of *Plasmodium hexamerium* infections in nestling and fledgling Starlings and Meadowlarks.

Figure 5. Proportions of the various life cycle stages of *Plasmodium hexamerium* found in the circulating blood of Starlings and Meadowlarks.

S1-3 - Segmenters with 1-3 merozoites.

S4-6 - Segmenters with 4-6 merozoites.

T - Immature trophozoites and ring stages.

G - Gametocytes.

Morning - Before 10:00 A.M.

Evening - After 4:00 P.M.

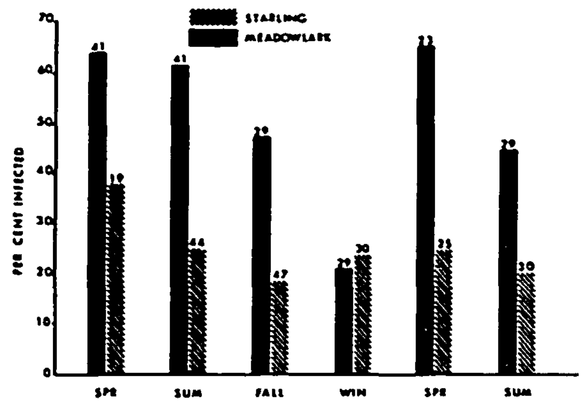


FIGURE 2

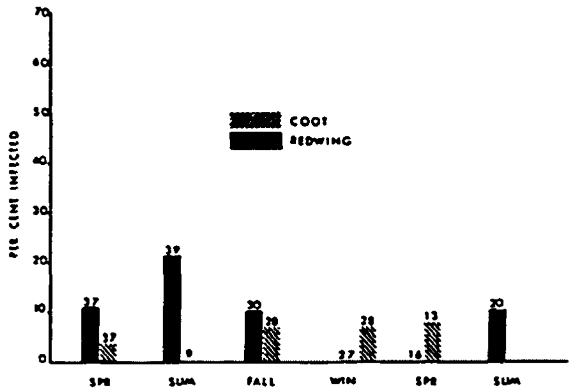


FIGURE 3

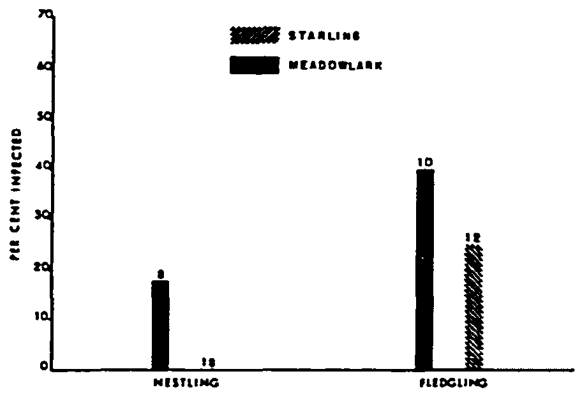


FIGURE 4

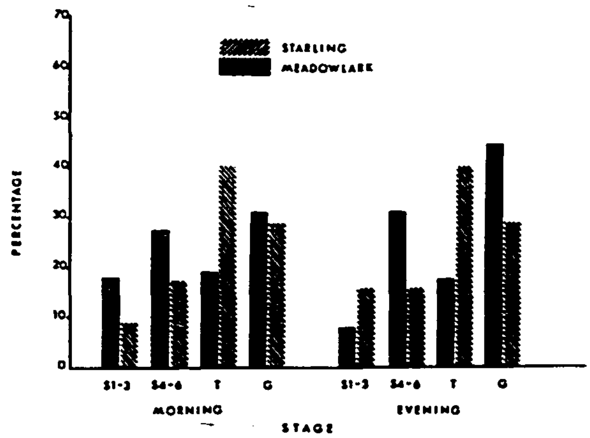


FIGURE 5

EXPLANATION OF FIGURES (continued)

Figure 6. *Plasmodium hexamerium*, Meadowlark Strain

Figure 7. *Plasmodium hexamerium*, Starling Strain

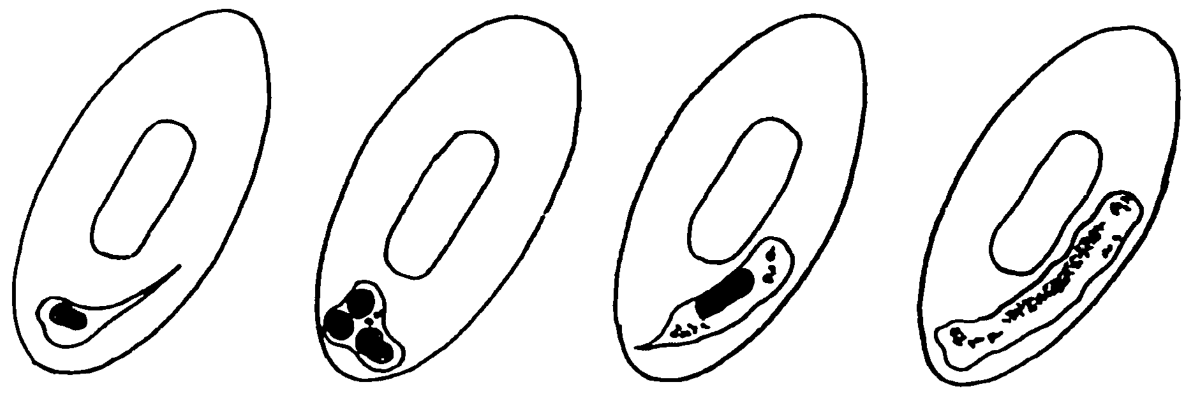
Figure 8. *Plasmodium* sp. (*P. lophurae* ?) from the American Coot.

a. Immature trophozoite

b. Mature segmenter

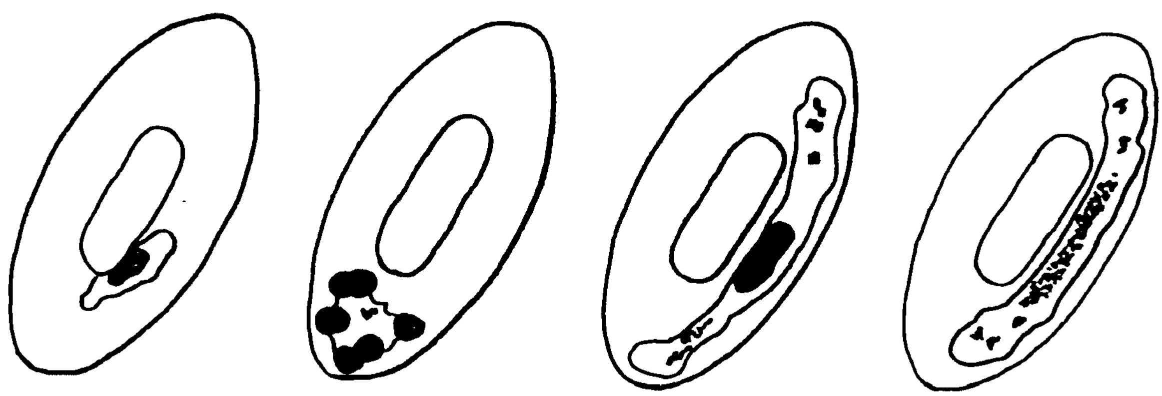
c. Macrogametocyte

d. Microgametocyte



a. b. c. d.

FIGURE 6 - PLASMODIUM HEXAMERIUM, MEADOWLARK STRAIN



a. b. c. d.

FIGURE 7 - PLASMODIUM HEXAMERIUM, STARLING STRAIN

a. b. c. d.

FIGURE 8 - PLASMODIUM SP. (P. LOPHURAE ?), FROM THE COOT

EXPLANATION OF FIGURES (continued)

Figure 9. Mosquitoes captured in traps baited with Starlings. Total catch was 115 mosquitoes in 12 trap-nights.

Figure 10. Relative abundance of Cheyenne Bottoms mosquitoes.

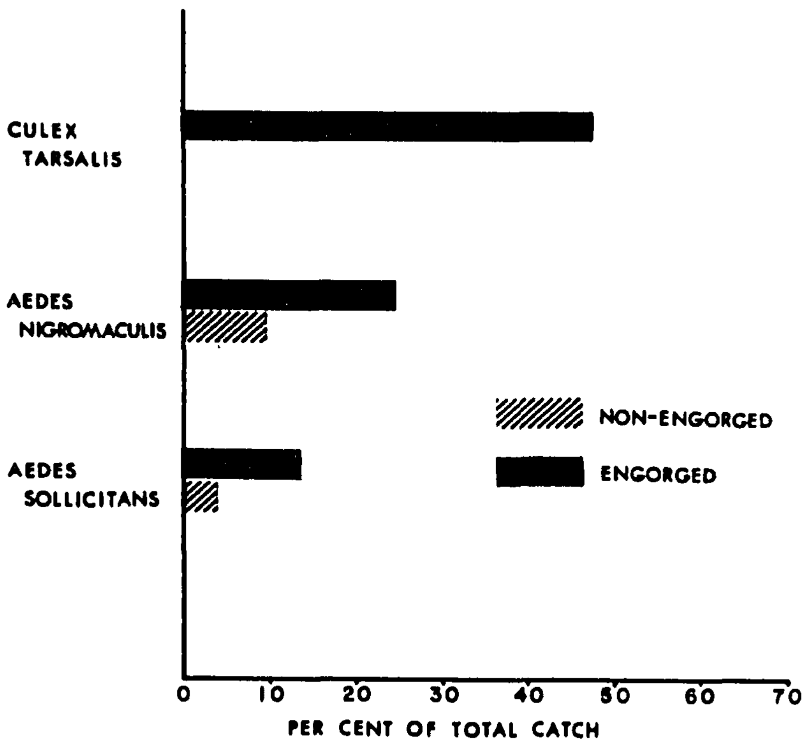


FIGURE 9

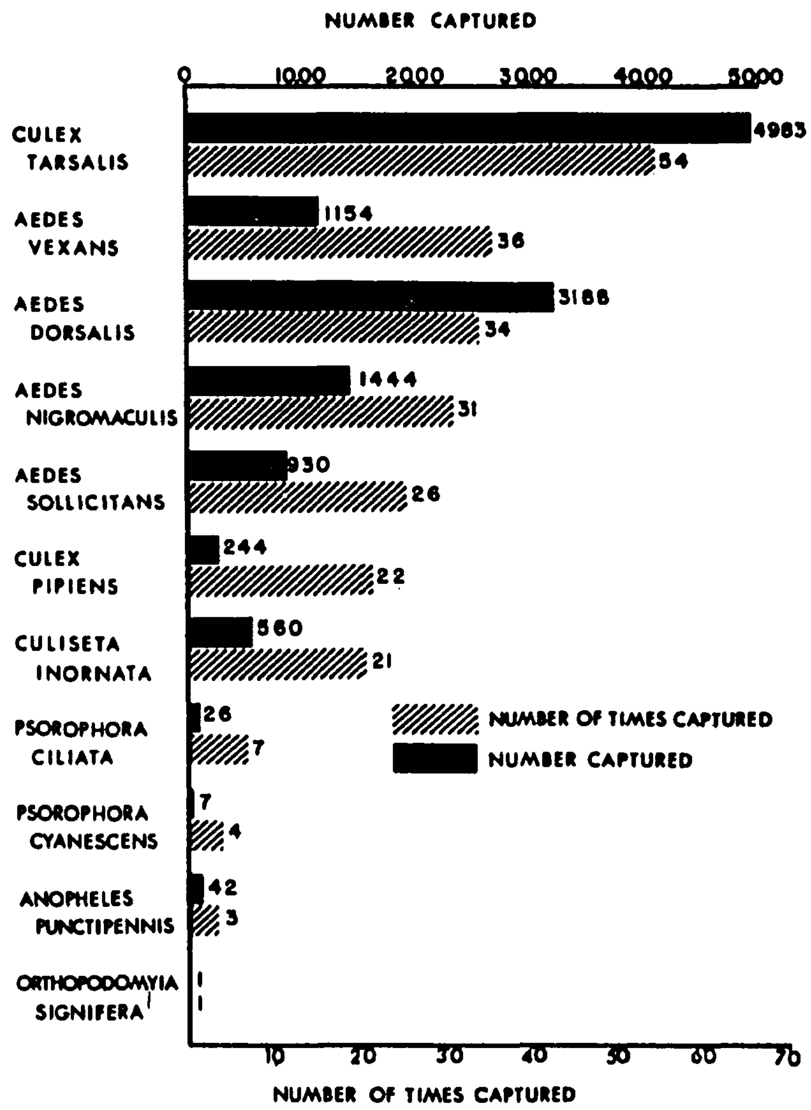


FIGURE 10

EXPLANATION OF FIGURES (continued)

Figure 11. Monthly fluctuations in overall mosquito abundance.

Figure 12. Relative population fluctuations of _Culex tarsalis_, _Culex pipiens_, and _Culiseta inornata_.

Figure 13. Relative population fluctuations of _Aedes nigromaculis_ and _A. sollicitans_.

Figure 14. Relative population fluctuations of _Aedes dorsalis_ and _A. vexans_.

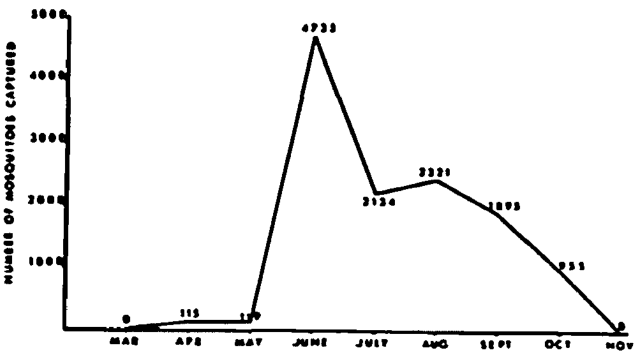


FIGURE 11

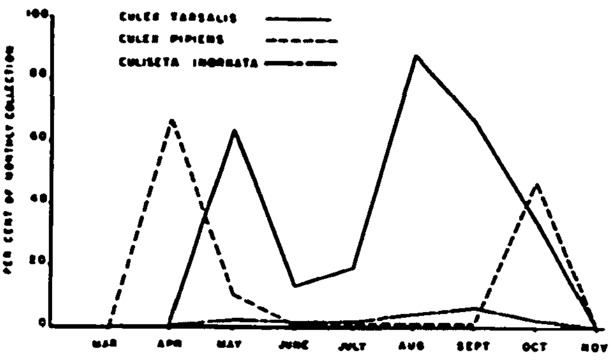


FIGURE 12

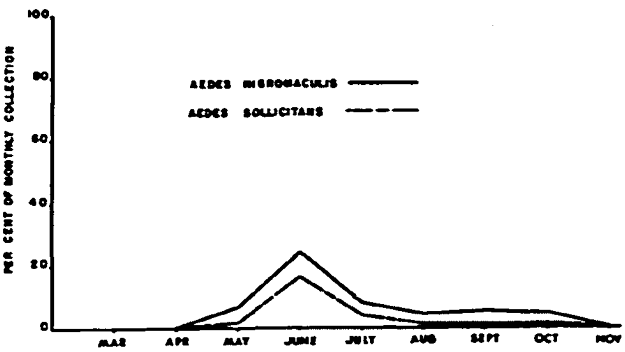


FIGURE 13

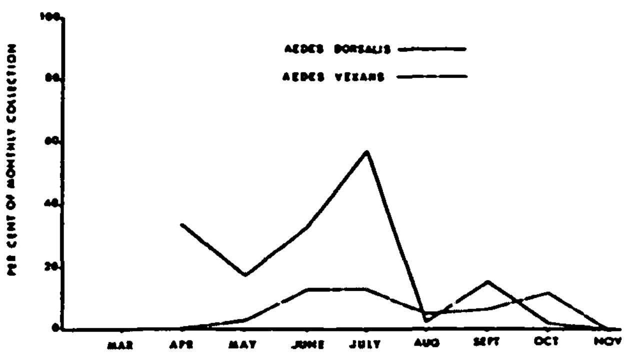


FIGURE 14