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**AVIAN MALARIA AMONG HOUSE SPARROWS: A SURVEY OF  
DISEASE AND MOSQUITO VECTORS (DIPTERA: CULICIDAE) IN  
REED CITY, MICHIGAN**

Luanne R. Gogolin<sup>1</sup> and Jerome E. Freier<sup>2</sup>

ABSTRACT

Nine of 350 house sparrows caught in Reed City, Michigan, had malaria parasites detectable on Giemsa-stained thin films. All of the infected birds were juveniles. Parasitemias were too low to permit identification of the *Plasmodium* species present. Collection of potential vector mosquitoes showed that *Culex pipiens* and *Culex restuans* were present, but in low numbers.

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The parasite causing malaria in birds was first described nearly 100 years ago by Danilewsky (1891) and, since that time, this organism has been the subject of numerous laboratory studies. Although early investigations involved the cultivation of the avian *Plasmodium* by direct blood passage from bird to bird, it was not until Ross's (1897) discovery of mosquito transmission that the full life cycle of avian malaria could be realized. For many years, studies have employed avian malaria as a model system for experimental work that might provide information relevant to the problems associated with human malaria. Investigations concentrating on the field aspects of avian malaria have been few in comparison with the number of laboratory studies conducted. Many of the early field studies were aimed at determining the seasonal incidence and geographic distribution of these parasites. However, most field studies have generally not taken into consideration the role of mosquito vectors in the natural maintenance of avian malaria in endemic foci; therefore, determination of field infection rates among mosquitoes vectors was seldom attempted.

The species of *Plasmodium* that cause avian malaria have been divided into four subgenera; however, only those involving birds in the order Passeriformes will be discussed in this report. Malaria among passerine birds is widely distributed throughout the tropical, subtropical, and temperate regions of the world. It is found on every continent and in nearly every country of the world (Garnham 1966). Migratory flights of birds have helped to bring about this cosmopolitan distribution. In North America, nine species of *Plasmodium* have been described from natural infections of birds. These are *P. cathemerium*, *P. circumflexum*, *P. elongatum*, *P. hexamerium*, *P. nucleophilum*, *P. polare*, *P. relictum*, *P. rouxi*, and *P. vaughani*. However, only *P. relictum*, *P. elongatum*, and *P. circumflexum* have been reported in passerine birds from Michigan (Stuht 1979). Once the parasite has been introduced into an area, the establishment of a transmission cycle depends on the presence of a suitable vector. The species of *Plasmodium* found in Michigan are all transmitted by culicine mosquitoes of the genus *Culex*. Species of *Culex*

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mosquitoes found in Michigan include *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cx. tarsalis*, *Cs. territans*, *Cx. erraticus*, and *Cx. peccator* (Cassani and Newson 1980). The first six of these species are known natural vectors of at least one or more species of avian malaria (Garnham 1966). In addition, natural avian hosts for passerine malaria are extensive; 109 species of birds have been found naturally infected with *P. relictum* alone. Thus, it is likely that many migratory and nonmigratory birds in Michigan might serve as suitable hosts for locally occurring species of *Plasmodium*.

The purpose of the present study was to determine the overall prevalence and seasonal incidence of avian malaria in a small town in west-central Michigan. We wished to observe the population dynamics of avian malaria parasites among a nonmigratory bird population in relation to the species composition and population density of mosquitoes that may be acting as vectors. The house sparrow, *Passer domesticus*, was selected for study because it is nonmigratory and has been shown to be an excellent reservoir host for avian malaria parasites (Herman et al. 1954). Also, house sparrows and *Culex* mosquitoes have been shown to be associated with the maintenance cycle of St. Louis encephalitis virus (McLean and Bowen 1980, Mitchell, et al. 1980). It was hoped that information from this study in the form of a field infection rate for parasites among vector mosquitoes and the incidence rate of this disease among house sparrows might be developed as a relative measure of mosquito vector competence. Such a factor could then be applied, in a predictive way, to those components of the natural transmission cycle of St. Louis encephalitis virus that relate to vector efficiency.

#### MATERIALS AND METHODS

**Study Site.** Reed City is located in Richmond Township of Osceola County (lat. 43°45' N long. 85°30' W; T7N, R10W). The city limits of Reed City occupy 453 ha and the human population in 1980 was 2221. The town is surrounded by agricultural lands, mostly dairy farms, that are under varying intensities of crop cultivation. Active gas and oil wells are located north of the city limits. In addition, several small lakes and streams are in the immediate vicinity and the Hersey River flows through the northeast section of the city. Specific study sites were selected along and on either side of a transect line extending from the northwest to the southeast corner of the city's boundaries. Eight sites (Fig. 1) were selected for the capturing of house sparrows and five sites were chosen for trapping mosquitoes. The exact location of each site depended upon identifying those residences containing habitats that appeared to be most suitable for both house sparrows and *Culex* mosquitoes. This study was conducted between 14 June and 12 September 1980.

Meteorological data for Reed City and the surrounding area was obtained from the U.S. Weather Station at Houghton Lake, Michigan.

**Birds.** House sparrows were captured using rigid walk-in traps (Tomahawk Trap Company) that were baited with cracked corn. These traps are designed specifically for house sparrows and only rarely are other species of birds caught in them. The house sparrow population was sampled three times each week on Monday, Wednesday, and Friday. Birds were allowed to enter the traps from 1600 h on the afternoon prior to the sampling day until 0900 h on the following morning. After each bird was removed from the trap, 0.1 ml of blood was withdrawn by veinipuncture of the jugular vein and a thin blood smear prepared. Each slide was coded according to the date of collection and location of the site. The thin blood smears were allowed to air dry before being transported to the laboratory where they were fixed for 10 min in absolute methanol and then stained for 25 min with Giemsa stain (Harleco Stains, diluted 1:20 in distilled water). Before release, the age and sex of each house sparrow was determined on the basis of plumage (Robbins et al. 1966) and skull ossification (Sudia et al. 1972, Summers-Smith 1963). Birds were also examined for the presence of ectoparasites. Before being released, each captured bird was marked with a non-toxic acrylic paint that was applied to the feathers on the throat region.

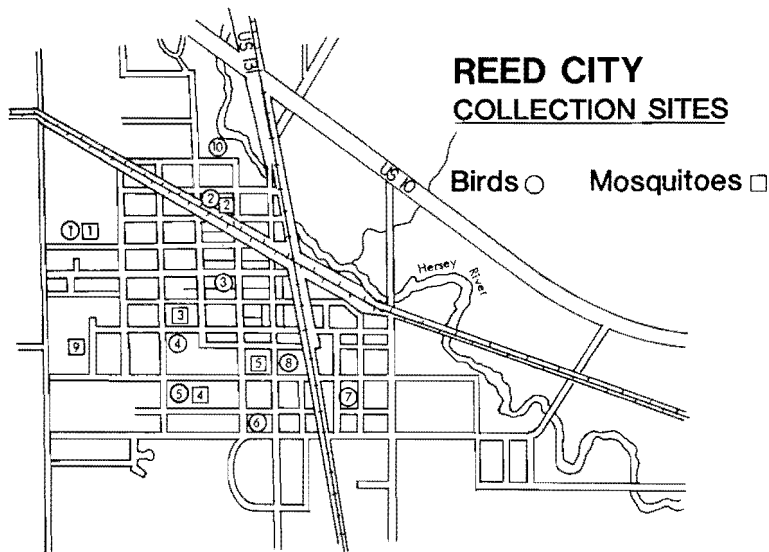


Figure 1. Sites for the collection of house sparrows (○) and mosquitoes (□) in Reed City, Michigan.

**Mosquitoes.** Mosquitoes were caught with CDC-miniature light traps that were operated three times per week on Sunday, Tuesday, and Thursday evenings. Specimen bags were removed by 0900 h on the following morning. Bags containing live mosquitoes were transported to the laboratory in an insulated container refrigerated with a synthetic coolant. Mosquito specimens were sorted on a chill table and the species identity of each individual determined (Stojanovich 1961). Female *Culex* spp. were placed separately in vials and stored at  $-70^{\circ}\text{C}$  until they could be examined for the presence of mosquito stages of malaria parasites.

**Parasites.** The presence of blood stages of malaria parasites was determined by microscopic examination of the Giemsa stained thin blood smears with a 100X panachromatic oil immersion objective. Each slide was screened for the presence of parasites by examining a minimum of 50,000 erythrocytes before a smear was considered negative. If any of the blood stages of malaria parasites were observed, the percentage parasitemia was determined by counting directly the number of parasites observed per an estimated 50,000 erythrocytes. Therefore, the level of infection for the blood sample obtained was expressed as a percentage of the erythrocytes examined.

Infection of mosquitoes by avian malaria parasites was determined by dissecting the midgut and salivary glands from each female in a drop of physiological saline. After placing a cover glass over the dissected organs, they were examined at a 200X magnification using phase contrast optics to reveal the presence of oocysts on the midgut or sporozoites in the salivary glands.

## RESULTS

A total of 350 house sparrows was captured and bled from the eight sites studied in Reed City. The age and sex distribution of the birds sampled is shown in Table 1. Juvenile birds of both sexes constituted 78.3% of the overall collection. Also, both sexes of juvenile birds seemed to be about equally likely to be captured in the traps since the male:female sex ratio among the birds caught was 1.06:1. However, among the adult

Table 1. Seasonal distribution of each age and sex of house sparrows captured.

Collection interval	Total no. birds	(%)	No. each age and sex <sup>a</sup>				
			JM	JF	AM	AF	
June	14-20	44	(12.6)	16	16	7	5
	21-27	24	(6.9)	8	7	9	0
	28- 4	37	(10.6)	19	7	6	5
July	5-11	64	(18.3)	20	27	10	7
	12-18	45	(12.8)	15	18	6	6
	19-25	33	(9.4)	15	16	1	1
	26- 1	23	(6.5)	12	8	2	1
August	2- 8	20	(5.7)	10	7	2	1
	9-15	15	(4.3)	5	5	2	3
	16-22	25	(6.9)	11	11	2	1
	23-29	4	(1.1)	2	2	0	0
	30- 5	8	(2.3)	5	3	0	0
Sept.	6-12	9	(2.6)	3	6	0	0
Totals	350		141	133	47	29	

<sup>a</sup>JM—juvenile male, JF—juvenile female, AM—adult male, AF—adult female.

Table 2. Distribution of mosquito species caught in light traps from each site.

Mosquito species	Site number					Totals
	1	2	3	4	5	
<i>Aedes canadensis</i>	9	2	7	8	4	30
<i>Aedes dorsalis</i>	1	4	10	4	5	24
<i>Aedes sollicitans</i>	4	5	0	0	1	10
<i>Aedes stimulans</i>	15	10	24	16	19	84
<i>Aedes vexans</i>	46	55	119	49	67	336
<i>Aedes</i> species	6	2	4	1	2	15
<i>Anopheles punctipennis</i>	3	10	10	6	11	40
<i>Anopheles quadrimaculatus</i>	1	2	3	2	9	17
<i>Culex pipiens</i>	1	1	8	2	8	20
<i>Culex restuans</i>	0	0	4	1	0	5
Totals	86	91	189	89	126	581

birds, more males than females were captured (male:female sex ratio of 1.62:1). During the entire collection season, only 10 house sparrows were recaptured and all of them were juvenile birds. The number of birds from either age group caught in the traps during each collection interval tended to vary in a cyclic manner. The collection peak was between 5-11 July. Although, when the data in Table 1 are plotted (not shown), smaller peaks were also observed for 14-20 June and 16-22 August. Also, no ectoparasites were found on any of the house sparrows captured.

From the five sites sampled, a total of 581 mosquitoes was collected. These specimens represented nine different species. Table 2 shows the distribution of mosquito species caught in light traps from each site. *Aedes vexans* and *Ae. stimulans* were the two most

Table 3. Seasonal distribution of mosquito species caught in light traps.

Mosquito species	Collection interval														Total collected
	June			July				August				Sept.			
	14-20	21-27	28-4	5-11	12-18	19-25	26-1	2-8	9-15	16-22	23-29	30-5	6-12		
<i>Aedes canadensis</i>	0	5	2	4	4	9	3	3	0	0	0	0	0	30	
<i>dorsalis</i>	0	1	2	4	7	10	0	0	0	0	0	0	0	24	
<i>solicitans</i>	0	1	3	1	2	3	0	0	0	0	0	0	0	10	
<i>stimulans</i>	5	14	18	14	10	10	5	1	1	1	2	2	1	84	
<i>vexans</i>	10	9	10	32	29	90	66	33	15	6	8	17	11	336	
species	0	3	1	5	3	1	0	1	1	0	0	0	0	15	
<i>Anopheles punctipennis</i>	2	2	1	5	1	7	3	2	5	1	3	3	5	40	
<i>quadrimaculatus</i>	0	0	3	3	4	1	0	2	3	0	1	0	0	17	
<i>Culex pipiens</i>	0	1	2	1	6	4	0	1	2	1	0	1	1	20	
<i>restuans</i>	1	0	0	0	0	0	0	2	1	1	0	0	0	5	
Species totals for each week	18	36	42	69	66	135	77	45	28	10	14	23	18	581	

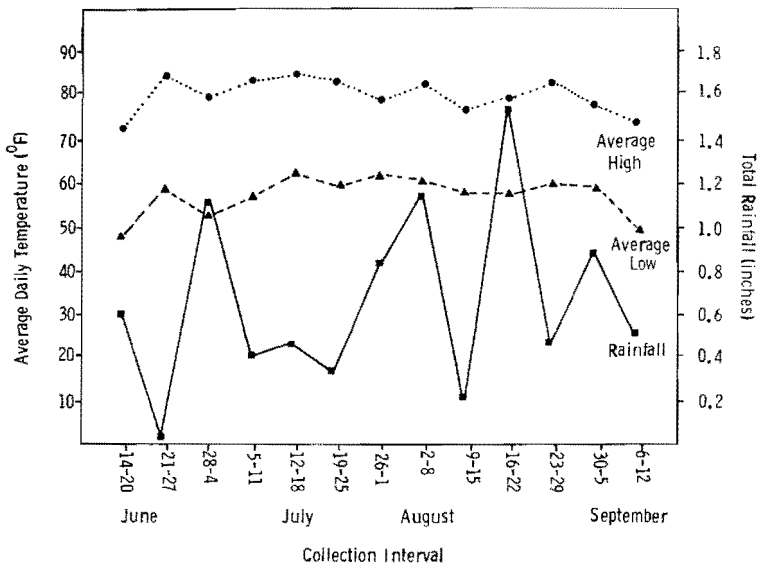


Figure 2. Average daily temperature (°F) and total rainfall (in.) for the vicinity of Reed City, Michigan, between 14 June and 12 September 1980.

predominant species comprising 57.8% and 14.5% of the total collection respectively. Of the potential vectors of avian malaria, 20 *Cx. pipiens* and five *Cx. restuans* accounted for 4.3% of the mosquitoes caught. Except for site number 3, the number of mosquitoes and distribution of species among the collection sites were similar. Site number 3 had approximately twice as many mosquitoes as any other individual site and this was due to

a large abundance of *Ae. vexans* mosquitoes. The majority (80%) of the *Culex* species collected were from sites 3 and 5.

The seasonal distribution of the mosquitoes caught is shown in Table 3. The overall number of mosquitoes caught increased gradually from 14 June until reaching peak during the collection interval of 19–25 July and then the number steadily declined for the remainder of the season. For each species, the seasonal distribution varied. *Aedes stimulans*, *Ae. canadensis*, *Ae. dorsalis*, and *Ae. sollicitans* were most abundant during the first 6 weeks of the season. However, *Ae. vexans* was most abundant during the last 8 weeks of the season. *Anopheles punctipennis* and *An. quadrimaculatus* were caught in low numbers throughout most of the summer. Although the number of *Culex* species collected was small, about one to six specimens were collected each week. Forty percent of the total *Culex* collection was obtained between 12–25 July.

The meteorological conditions during the summer of 1980 are shown in Figure 2. The average daily low and high temperatures extended between 15°C and 28°C for most of the collection season. There were no prolonged periods of unseasonably cold weather that might adversely affect mosquito activity. However, heavy rainfall did occur periodically during the season with the greatest amounts falling during 28 June–4 July, 2–8 August, and 16–22 August intervals. While some reduction in the numbers of mosquitoes caught was noted during the weeks with heavy rainfall, the differences were due primarily to a decrease in the number of *Ae. vexans* mosquitoes entering the traps. Three weeks following the heavy rainfall between 28 June and 4 July, *Ae. vexans*, the only floodwater mosquito species collected, reached a peak in the number caught.

Microscopic examination of Giemsa stained thin films showed (Table 4) that nine of 350 birds tested (2.6%) were infected with *Plasmodium* parasites. The first infections were observed in late June and early July, but the highest prevalence (67%) was detected from middle to late August. All of the infections observed were from juvenile birds; however, the distribution of infections between sexes was nearly equal. Except for an infected house sparrow collected on 30 June with a parasitemia of 8.3%, all of the other infection levels were less than 1.0%. Identification of the *Plasmodium* species obtained from the birds tested in this study was not possible because the number of parasites and stages of development needed were insufficient to establish identities with certainty. Infected house sparrows were caught at five of the eight collection sites with site 8 yielding three infected birds, each of the birds was caught approximately 10–14 days apart from 13 August to 10

Table 4. *Plasmodium* infections in house sparrows captured and examined from each site.

Site	No. examined	No. positive	(%)	Percentage parasitemia	Age-Sex <sup>a</sup>	Date of collection
1	80	1	(1.3)	13.00	JF	30 June
2	37	0				
3	24	2	(8.3)	0.02	JM	18 August
				0.20	JM	18 August
4	27	1	(3.7)	0.06	JM	4 July
5	7	0				
6	47	2	(4.2)	0.01	JM	18 August
				0.07	JF	27 August
7	73	0				
8	55	3	(5.4)	0.01	JF	15 August
				0.02	JF	29 August
				0.01	JF	10 September
Totals	350	9	(2.6)			

<sup>a</sup>JM—juvenile male; JF—juvenile female.

September. From the five sites in which infected birds were captured, the house sparrow infection rates in relation to the total sample size from each site were quite similar.

Although only 25 *Culex* mosquitoes were caught in this study, each one was dissected and tissues of the midgut and salivary glands examined for the presence of malaria parasites. No ookinetes were observed on the exterior of the midgut, nor were sporozoites detected in any of the salivary glands examined from any of these mosquitoes.

## DISCUSSION

This study has demonstrated that avian malaria does occur among house sparrows in Reed City, Michigan. The overall infection rate that we observed was 2.6%; however, it is likely that the actual rate of *Plasmodium* infection is greater than this since many birds may have inapparent infections not detectable on a single thin blood smear. Herman et al. (1954) found that on the basis of a time course analysis of blood smears prepared at periodic intervals after infection that parasites were evident in peripheral circulation for only about 24 h. Thus, many infections may be missed by a single blood smear obtained from a bird at a given point in time. In addition to a parasitemia following the initial infection, avian hosts frequently have relapses with recurring parasitemias, making it difficult to pinpoint when a bird first became infected. However, because all of the birds found infected in this study were juveniles, it is assumed at least that these infections were obtained during the summer of 1980.

In other studies of naturally occurring *Plasmodium* infections among house sparrows, rates of infection have been relatively high. For example, Micks (1949) found an overall incidence of 13.8% in 210 house sparrows tested in Maryland and Jordan (1943) found 16.7% of 418 house sparrows examined in Athens, Georgia, to be infected. In house sparrows caught either in or near Syracuse, New York, Manwell and Herman (1935) found seven of 245 (2.9%) infected with malaria parasites. In the detailed studies of Herman et al. (1954) in Kern County, California, the incidence of infection was shown to vary considerably from year to year and in relation to the type of habitat utilized by the house sparrows. Rural areas containing a predominance of either orchards or cultivated fields had *Plasmodium* infections as high as 40%. In contrast, house sparrows from urban habitats had a substantially lower infection rate of 3.2%. This rate more closely approximated the one that we observed for Reed City, Michigan.

As reported by Herman et al. (1954), we also observed a seasonal peak in the incidence of *Plasmodium* infections which occurred in July and August. In our study, the first appearance of malaria parasites in the blood of house sparrows was during the last week of June. It should be noted that following the introduction of sporozoites from an infected mosquito into the avian host, a period of prepatency occurs which lasts from several days to a few weeks. Therefore, the greatest incidence of infection among the house sparrows would be expected to occur soon after the greatest amount of *Culex* activity. About three-fourths of the house sparrows that we found infected were collected between 9 and 15 August. Unfortunately, the numbers of *Culex* mosquitoes collected were too few to permit the determination of seasonal peaks.

Of the eight species of *Culex* mosquitoes known to occur in Michigan, six have been shown to be either natural or experimental vectors of avian malaria (Garnham 1966). However, only *Cx. pipiens* and *Cx. restuans* were collected in the Reed City study site. This does not exclude other species of *Culex* from being present since the light traps used for mosquito collection may not have been attractive to other species in this genus. Other surveys of mosquitoes in urban areas in southern Michigan, using larval collections intended to determine species composition of an area, have shown *Cx. pipiens* and *Cx. restuans* to be the predominant *Culex* species in most urban and suburban habitats (McGroarty et al. 1976).

The number of *Culex* mosquitoes caught in this study represents less than 5% of the total number of mosquitoes caught. This low density of potential vectors that we observed may have been due to the placement of traps in inappropriate locations or the natural population



of *Culex* mosquitoes may have been low due to an insufficient number of larval breeding sites to support a large population. Although the presence of potential *Culex* breeding sites was determined at the beginning of this study, no attempt was made to determine the existence or number of *Culex* mosquitoes in these larval sites.

To determine whether the low density of *Culex* mosquitoes was responsible for the low level of *Plasmodium* infections among the house sparrow population would require additional observations over several seasons. Also, further analysis of the population dynamics of avian malaria in relation to mosquito vector populations should involve additional study sites for a comparative analysis. Both urban and rural sites in areas with a varying abundance of *Culex* mosquitoes might be more desirable for further studies.

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