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Spring Relapse of *Plasmodium relictum* Infections in an Experimental Field Population of English Sparrows (*Passer domesticus*) *

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Abstract

A field population of English sparrows (*Passer domesticus*) was used to test the hypothesis that the reported spring maximum in the prevalence of *Plasmodium* infections is the result of an elevation of circulating parasite populations in established infections. Sparrows were captured, banded, infected with *Plasmodium relictum*, and released. Since natural infections were negligible in this population, patent infections in recaptured birds were assumed to result from the inoculation of parasites. The primary parasitemia in the experimental birds lasted approximately 25 days, while a substantial proportion of low intensity infections persisted through 125 days. Analysis of the seasonal distribution of patent infections in birds infected 125 days or longer showed a sharp rise in both prevalence and parasitemia in the spring. This study thus supports the hypothesis that spring relapse of established infections is responsible for the high spring prevalence of *Plasmodium* in birds.

Introduction

In surveys of malarial parasites of birds, the highest prevalence of patent infections consistently occurs in the spring of the year.[1,2,8,10,11] This phenomenon in *Leucocytozoon* infections has been attributed to relapse of established infections rather than a spring maximum in transmission.[4,7] Several investigators have suggested that a similar spring relapse phenomenon exists in *Plasmodium*,[2,8,9] but experimental investigation of this hypothesis is needed. The present study utilizes an experimental field population to explore the possibility that the spring maximum in prevalence of *Plasmodium* is the result of an elevation of parasite populations in established infections.

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The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, NAS-NRC.

The opinions and assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

Materials and Methods

The study reported here was carried out on a 5-acre area of the horse barns on the agriculture farm of the Pennsylvania State University, University Park, Pennsylvania. The experimental host was the English sparrow *(Passer domesticus)* and the parasite was *Plasmodium relictum* (Grassi and Feletti, 1891). The specific isolates used in this study were obtained by Dr. Edith Box in Galveston, Texas; and by the author in State College, Pennsylvania. Both isolates were known to relapse and to infect English sparrows. All experimental infections were induced by subinoculation of whole blood in the jugular vein using a 1 ml heparin-rinsed tuberculin syringe fitted with a 27-gauge needle. The size of the parasite inoculum was not standardized.

Thin blood films were prepared from blood collected by clipping the toenail of an experimental bird. The films were fixed in methyl alcohol, stained in Giemsa's stain and examined under oil immersion optics (970X). Each slide was examined 10 minutes. Approximately 80,000 red blood cells were scanned in each film by this protocol.

Sparrows were captured using Japanese mist nets. Isodiagnosis was by subinoculation of 0.2 ml whole blood into uninfected canaries or sparrows. Blood films from recipients were prepared and examined semiweekly for six weeks.

Statistical analyses were made using the X^2 test.

Experimental Design and Results

A wild population of English sparrows was used to study the problem of seasonal relapse. Malaria infections, detectable with the methods used, were uncommon in this population (Table 1). The experimental design involved capturing sparrows from this population, banding them, infecting them with *Plasmodium relictum*, releasing them, and monitoring infections in recaptured birds. Isodiagnosis of birds handled in this manner verified that our transmission techniques resulted in a high proportion of infected birds (28 of 30, or 93.3% demonstrably positive).

Continual screening of blood films from uninoculated birds (Table 1) demonstrated that infections by mosquito transmission remained negligible throughout the study. Patent infections in recaptured birds were therefore assumed to be the product of the experimental introduction of parasites at the time of initial capture. Seasonal changes in parasite populations in established infections could therefore be monitored without the complicating effect of natural transmission.

During the course of this study, March 1967 through July 1968, 610 individual sparrows were inoculated, banded and released. A total of 479 recaptures of 332 banded birds occurred during the same period.

Two parameters were used to measure parasite populations in this study: prevalence — the percentage of birds with patent infections; and parasitemia — the number of parasites per 100,000 RBC's in the sample of recaptured birds. Of initial interest was the distribution of these parameters with respect to the time interval from day of infections to day of recapture (infection interval) (Fig. 1). Parasitemia in this population was greatest in the 10-20-day interval and then declined rapidly. These data agree with previous investigators who estimated the duration of the primary attack for this parasite to be approximately 15-25 days.[3,9,12] Prevalence data reveal a quite different picture (Fig. 1). Between 50% and 55% of the birds had patent infections through 40 days. Prevalence then declined gradually through about 125 days. These data suggest that patent infections through day 125 were associated with the primary attack. For this reason, consideration of parasite population changes in established infections includes only birds infected 125 days or longer.

Analysis of the seasonal distribution of patent infections in birds with established infections reveals a sharp trend to patency in the spring of the year (Fig. 2).

TABLE 1. *Monthly prevalence of* Plasmodium *infections in a population of English sparrows at University Park, Pennsylvania; March, 1967 through July, 1968. Data refer only to birds at the time of initial capture, prior to their experimental infection.*

Month	Proportion of adults with patent infections	Proportion of young with patent infections	Proportion of unknown aged birds with patent infections	% of total birds with patent infections
Mar, 1967	0/40	——	——	0
Apr	0/74	——	——	0
May	0/64	0/11	——	0
Jun	0/49	1/8	——	1.8
Jul	0/18	1/9	——	3.7
Aug	0/1	0/40	——	0
Sep	0/1	2/47	0/1	4.2
Oct	0/4	0/12	0/1	0
Nov	——	——	0/36	0
Dec	——	——	0/52	0
Jan, 1968	1/7	——	——	14.3
Feb	0/16	——	——	0
Mar	0/29	——	——	0
Apr	0/24	——	——	0
May	0/12	0/4	——	0
Jun	0/3	0/2	——	0
Jul	0/9	4/37	——	8.7
	1/351	8/170	0/90	1.5

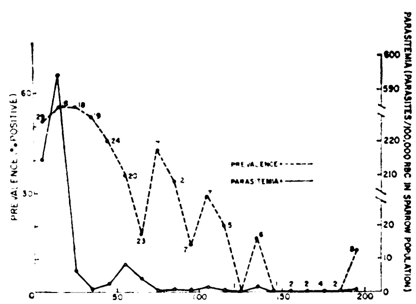

FIGURE 1. *The distribution of patent infections with respect to infection interval (number of days from day of infection to day of rectpaure) for sparrows experimentally infected with* P. relictum. *Data included in this graph were collected from March 1967 through Feb-ruary 1968. Numbers indicate sample size.*

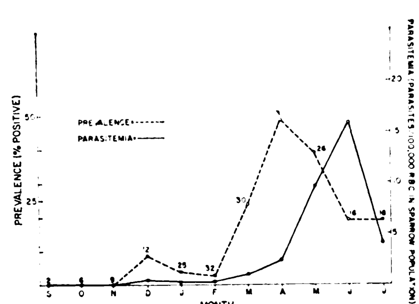

FIGURE 2. *Seasonal distribution of patent infections in sparrows infected more than 125 days. Numbers indicate sample size.*

Combining monthly prevalence data, the following seasonal figures were obtained: none of 17 birds had patent infections in the fall (September-November); 4.3% of 69 birds had patent infections in the winter (December-February); 36.8% of 87 birds had patent infections in the spring (March-May); and 18.7% of 32 birds had patent infections in early summer (June-July). These seasonal differences in prevalence are statistically significant ($P < 0.001$). Worthy of re-emphasis is the fact that none of 74 control birds had patent infections during the March-June period of 1968 (Table 1), indicating that patent infections in recaptured birds were renewed manifestations of established infections.

Parasitemia levels also showed seasonal variation. Parasitemias never exceeded one parasite in 100,000 RBC's until April, and then increased to a maximum of 16.6 parasites in 100,000 RBC's in June. Of interest is the relationship between prevalence and parasitemia during the spring and early summer. Early in the spring a high proportion of the birds had patent infections, but very few parasites were found in each case. As the season advanced there were fewer patent birds but the parasitemias in these infections were much heavier.

When the effect of infection interval is considered (Table 2), it is apparent that patency in established infections is favored by a shorter infection interval ($P < .001$). Nonetheless, patent infections in spring were found in every infection interval class. The effect of season is apparent when prevalence in fall and winter is compared with prevalence in spring within each infection interval class ($P < .001$ for 125-199 class and $P < .001$ for combined 200-499 class).

Female sparrows had a higher prevalence of patent infections than males in the spring (Table 3 — $P < .05$). The difference was most evident in April and May but the situation may be reversed in March. Available data did not permit statistical analyses of individual months, but they suggest that further investigation of this area might be fruitful.

TABLE 2. *Effect of infection interval (number of days from day of infection to day of recapture) and season on prevalence of patent infections in English sparrows.*

	Fall and Winter		Spring	
Infection interval (days)	No. birds examined	% with patent infections	No. birds examined	% with patent infections
125-199	24	8.3	28	71.4
200-299	52	1.9	14	28.6
300-399	9	0	40	15.0
400-499	0	—	5	40.0
	85	3.5	87	36.8

TABLE 3. *Effect of sex on prevalence of patent infections in English sparrows in spring.*

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Month	No. birds examined	% with patent infections	No. birds examined	% with patent infections
Mar	22	27.3	8	12.5
Apr	17	35.3	14	64.3
May	14	21.4	12	58.3
	53	28.3	34	50.0

Discussion

Data collected in this study indicate that populations of *Plasmodium relictum* in established infections tend to be elevated in the spring. Previous evidence for a spring relapse in *Plasmodium* has been based on field surveys while experimental evidence supporting this hypothesis has been unavailable, with one exception. Box[2] kept English sparrows in mosquito-proof cages in the laboratory and noted a spring maximum in the proportion of positive blood films taken from this population. The experimental design of the present study has the advantage of utilizing a wild population in its natural habitat. Methods of collecting blood samples were the same as those used in the surveys in which spring relapse was first suspected; that is, birds were simply captured, bled, and released. The data therefore represent parasite population changes in a host population which is experiencing ambient environmental conditions and negligible curtailment of normal seasonal behavior.

Data from subinoculation tests indicate that 93.3% of the birds inoculated and released were infected. Yet the highest prevalence of patent infections during the primary attack never exceeded 55.6% (Fig. 1). The difference between prevalence by isodiagnosis and prevalence by blood film analysis is significant for every infection interval class throughout the primary attack ($P < .01$). This difference may reflect the efficiency of the blood film technique as a method in blood parasite surveys of birds. Under the most optimum conditions (the first 30 days of the primary attack) blood film analysis revealed only 59.6% (55.6/93.3) of the estimated true prevalence. Under less than optimal conditions, the efficiency of this technique may be expected to decline even further. For example, the prevalence for birds infected for 50-150 days is 11.2%, or approximately 12% of the true prevalence. These data are in agreement with the contention of Herman *et al.*,[6] who believe that prevalence data in avian plasmodial surveys are considerably more accurate when obtained by the technique of isodiagnosis.

In April, the prevalence of patent infections in birds infected 125 days or longer was 48.3% (Fig. 2). This figure is not significantly different from the highest prevalence recorded during the primary attack ($0.50 < P < 0.70$), suggesting that the onset of spring relapse is a synchronous phenomenon affecting the entire population of infected hosts.

Coatney and Cooper[5] presented evidence indicating that the spring maximum of infections of *Plasmodium vivax* in man is dependent not on season but on a wave of relapses occurring at a fixed interval following infections. According to their interpretation, the infections relapse in spring merely because they were acquired at approximately the same time during the previous summer. In the present study on avian malaria, infections were induced throughout the year and spring relapses were noted in all infection interval classes (Table 2). Comparisons within infection interval classes showed that patent infections in spring could not be explained on the basis of an intrinsic rhythm in the parasite. It thus appears that spring relapse may be triggered by physiological changes in the host that occur in response to reasonal changes in the physical environment. The mechanism of this phenomenon presents an interesting topic for further investigation. Some of the observations of this study, such as the population-wide nature of spring relapse and the sexual differences in prevalence, may provide clues to a solution.

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