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Multispecies *Plasmodium* Infections of Humans

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Abstract

We analyzed point-prevalence data from 19 recent studies of human populations in which either *Plasmodium ovale* or *Plasmodium vivax* co-occur with *Plasmodium falciparum* and *Plasmodium malariae*. Although the only statistical interactions among sympatric congeners are pairwise, the frequencies of mixed-species infections relative to standard hypotheses of species sampling independence show no strong relation to overall malaria prevalence. The striking difference between the *P. falciparum*–*P. malariae*–*P. ovale* and the *P. falciparum*–*P. malariae*–*P. vivax* data is that the first typically shows a statistical surplus of mixed-species infections and the second a deficit. This suggests that the number of *Plasmodium* species present in a human population may be less important in determining the frequencies of mixed-species infections than is the identity of those species.

There are a number of conspicuous phenotypic as well as geographic differences among the 4 *Plasmodium* species that cause human malaria. The 3-day *Plasmodium malariae* erythrocytic schizogony cycle produces quartan rather than tertian fevers; *Plasmodium ovale* and *Plasmodium vivax* are said to invade only the youngest, *Plasmodium malariae* only the oldest, and *Plasmodium falciparum* all ages of erythrocytes; *P. vivax* is restricted to Duffy-positive erythrocytes; only *P. ovale* and *P. vivax* relapse by means of latent hypnozoites in the liver; only *P. falciparum* sequesters maturing gametocytes; each species differs in its typical asexual- and sexual-form densities and case-to-case generation times. Although the recorded history of human malaria implies that combinations of these 4 species have been in contact for tens of thousands of *Plasmodium* generations, the much higher case-fatality rates of *P. falciparum* have ensured it a virtual monopoly on scientific attention. Like our predecessors (Cohen, 1973; Molineaux et al., 1980; Richie, 1988), we suspect that significant clinical, epidemiological, and biological insights may emerge from studies of relationships between *P. falciparum* and its congeners, both in their own right and as bounding cases for phenotypic interactions among prospective recombinants.

A previous paper presented evidence that different pairs of *Plasmodium* species may show different patterns of joint dynamics (McKenzie and Bossert, 1997a). With the *P. falciparum*–*P. vivax* pair, for instance, a higher overall prevalence in humans is associated with fewer mixed-species infections than expected on the basis of the product of individual species prevalences, though this is not so with the *P. falciparum*–*P. malariae* pair. We have also shown that *Plasmodium* species may share not only *Anopheles* vector species but individual mosquitoes and that the patterns of mixed-species infections in mosquito populations may differ from those in corresponding human populations (McKenzie and Bossert, 1997b).

Here, we analyze reports from human populations in which either *P. ovale* or *P. vivax* co-occur with *P. falciparum* and *P. malariae*—the recent instances of polysympatry that most closely resemble those examined by our predecessors. Like them, we focus on interactions that may be reflected in the independence of species prevalences, largely because data on which to base more mechanistic causal hypotheses about relationships between *Plasmodium* infra- and suprapopulation dynamics are still lacking, even for single-species circumstances (McKenzie and Bossert, 1997c, 1998a, 1998b). Unlike our predecessors, we focus on possibilities of

distinct prevalence patterns and species-specific associations within and between the 2 sets of congeners.

MATERIALS AND METHODS

We surveyed English- and French-language epidemiological reports published from 1984 to 1997, inclusive, and available in Countway or Mayr libraries at Harvard University. The information we used was thus more recent than that used in Cohen's (1973) or Richie's (1988) analyses. Many recent studies report results only for *P. falciparum*, or for *P. falciparum* and 1 other species, even were other species present. Several reports contain unresolvable arithmetic inconsistencies. We analyzed only studies from which we could obtain complete, exact contingency tables, i.e., those that include figures for an uninfected category, explicitly report the presence or absence of multiple species and multispecies malaria infections, and if present explicitly distinguish the numbers and the species involved. As before (McKenzie and Bossert, 1997a), we analyzed only microscopy results, and we excluded studies that do not identify the period, place, and methods of sample collection. No study that reports 4 *Plasmodium* species met our criteria.

Our analytical objectives and methods again parallel those of our predecessors but with the costs and benefits that accrue from our use of exact data (see McKenzie and Bossert, 1997a). No study that reports 3 *Plasmodium* species and meets our criteria analyzes its findings with respect to multispecies infection. We extracted categorical point-prevalence data from each report to construct contingency tables, each containing counts of the presence and absence within individuals of each of the *Plasmodium* species reported present in the human population. Several tables contained 1 or more 0 entries: with 3 or fewer 0 entries in a table we added 0.5 to each cell to estimate the test statistic (Pagano and Gauvreau, 1993), and with 4 or more 0 entries in a table (as occurred in 2 tables), we eliminated it from further analysis. Though we considered the final tables too few for further spatial and temporal analysis, we did designate tables as before, as originating from a single village sampled within a single month (Level A), multiple villages sampled within a single month (Level B), a single village sampled over multiple months (Level C), or multiple villages sampled over multiple months (Level D). As before, we tested each table against standard null hypotheses of species sampling independence.

Tests of statistical independence are readily extended from 2-dimensional to multidimensional tables through log-linear models (Bishop et al., 1975; Christensen, 1990; Everitt, 1992): these form the discrete-multivariate analog to ANOVA and regression. At the top of the hierarchical sequence of models for 3-dimensional tables (here $2 \times 2 \times 2$), the most complex model is used to test for a 3-factor interaction, i.e., for a joint association between all 3 considered simultaneously. The simplest, lowest-level model in the hierarchy is used to test whether all 3 factors are completely independent of each other, i.e., essentially whether the prevalence of each of the possible multispecies infections is the product of the individual species prevalences. Between these 2 extremes is a series of 6 models encompassing the possible conditional 2-factor interactions between any factors A, B, and C: 3 models in the Bayesian form "A, B given C", and 3 in the form "A given B, C". For instance, here 1 of the first 3 conditional models is used to test whether *P. falciparum* and *P. malariae* are independent given the levels of the third species (*P. ovale* or *P. vivax*).

As before, because no marginal totals were fixed we used the *G*-test, with Williams' correction (Sokal and Rohlf, 1981), and we counted a table for which $P < 0.01$ significantly different from that expected. Because we had no basis for assumptions about any underlying distribution(s) of prevalences, we used the nonparametric Mann-Whitney test for distributional differences. As before (McKenzie and Bossert, 1997a), we used *G* and Mann-Whitney tests to check

whether distributions of low-valued cell counts (including zeroes), sample sizes, or clinical status biased each putative relationship; they did not.

RESULTS

Nineteen reports fulfill our criteria and describe prevalences of exactly 3 *Plasmodium* species. Twelve studies from Africa report the co-occurrence of *P. falciparum*, *P. malariae*, and *P. ovale*. Two studies from south Asia, 2 from southeast Asia, and 3 from the southwest Pacific report the co-occurrence of *P. falciparum*, *P. malariae*, and *P. vivax*. From these 19 reports, we extracted 29 contingency tables, sorted them according to the particular species present, then tested each for departures from species sampling independence. All tables contained sample sizes greater than 100.

Statistically significant departures from species sampling independence appeared in 11 tables overall (38%); see Table I. No table showed a significant 3-factor interaction. All significant tables implied departures from complete independence among the 3 species through conditional 2-factor interactions of the form “A, B given C”; 2 of the tables implied the presence of all 3 possible interactions of this form. Nonsignificant tables implied complete independence, hence no 2-way interactions. Table II summarizes the results for significant tables: although all interactions are pairwise, the only clear patterns among particular species pairs are the interactions between *P. falciparum* and *P. malariae* in 6 of the 7 significant *P. falciparum*–*P. malariae*–*P. ovale* tables and between *P. falciparum* and *P. vivax* in 3 of the 4 significant *P. falciparum*–*P. malariae*–*P. vivax* tables.

The 7 significant *P. falciparum*–*P. malariae*–*P. ovale* tables are scattered among the 4 spatial–temporal levels of analysis, in contrast to the complete correspondence between significant and level-D *P. falciparum*–*P. malariae*–*P. vivax* tables. Figure 1 shows distributional relationships between prevalence and G-test results: we found no distributional differences between significant and nonsignificant tables with either *P. falciparum*–*P. malariae*–*P. ovale* or *P. falciparum*–*P. malariae*–*P. vivax*.

To place these results in historical perspective, we extracted 32 complete, exact $2 \times 2 \times 2$ tables from the review by Knowles and White (1930). The underlying data appear generally comparable but should be viewed with some caution; the authors credit the many researchers who collected these data between 1911 and 1929 but do not always provide adequate details about their methods. Because *P. ovale* was seldom recognized as a distinct etiologic entity during this era, it is very likely that their classification of 2–3 tables (from Africa) as *P. falciparum*–*P. malariae*–*P. vivax* should be revised. However, because each of these fits the pattern of the rest (see below), excluding them had no substantial effect. As with the 1984–1997 data, no table showed a significant 3-factor interaction, and all significant tables implied conditional 2-factor interactions of the form “A, B given C”. Figure 2 shows distributional relationships between prevalence and G-test results for the 1911–1929 tables and again indicates no distributional differences between the significant and nonsignificant tables.

The 1911–1929 data also provide a novel perspective on 1 striking difference between the composition of the *P. falciparum*–*P. malariae*–*P. ovale* and the *P. falciparum*–*P. malariae*–*P. vivax* tables constructed from modern data: in modern *P. falciparum*–*P. malariae*–*P. ovale* tables, the observed frequencies in the single-species cells were generally lower and in the dual-species cells generally higher than those expected under complete independence, whereas the reverse was the typical pattern in *P. falciparum*–*P. malariae*–*P. vivax* tables. This contrast was most evident among significant tables, between single-species cells, among single- and dual-species cells involving *P. falciparum* in significant tables, and between modern *P. falciparum*–*P. malariae*–*P. ovale* and historical *P. falciparum*–*P. malariae*–*P.*

vivax. The species-pair interactions noted above correspond to surpluses of *P. falciparum*–*P. malariae* infections in the same 6 significant *P. falciparum*–*P. malariae*–*P. ovale* tables and deficits of *P. falciparum*–*P. vivax* infections in the same 3 significant *P. falciparum*–*P. malariae*–*P. vivax* tables. Figure 3 summarizes these observations. Figure 4 summarizes data from human populations with 2, 3, or 4 polysympatric *Plasmodium* species and suggests that neither overall malaria prevalence nor the fraction of malaria infections that are mixed-species relate simply to the number of species present.

DISCUSSION

The results presented here imply that the number of sympatric *Plasmodium* species in a human population may be a less important influence on the frequencies of mixed-species infections than is the identity of those species. Significant departures from the standard statistical hypothesis of species independence occur in 3-, as well as in 2-species, circumstances; each such departure depends on pairwise associations but not necessarily on the same species pair (s). Unlike 2-species circumstances, however, in both modern and historical 3-species data the statistical deficits or surpluses of mixed-species infections relative to standard null hypotheses of species independence show no particular relation to overall malaria prevalence. The remarkable difference between the *P. falciparum*–*P. malariae*–*P. ovale* and *P. falciparum*–*P. malariae*–*P. vivax* data is that the first more typically shows a statistical surplus of mixed-species infections, the second a deficit, a deficit that appears even more strongly in the relevant data from 1911 to 1929.

This distinction may have contributed to several disagreements among our predecessors. Cohen's (1973) conclusion that deficits of mixed-species infections were common was based on a review of 14 studies conducted between 1929 and 1964; 13 of these 14 studies were of *P. falciparum*–*P. malariae*–*P. vivax*. The surplus of mixed-species infections reported by Molineaux et al. (1980) and Molineaux and Gramiccia (1980) appeared in *P. falciparum*–*P. malariae*–*P. ovale* data collected during the 1969–1976 Garki project. Richie's (1988) conclusion that the prevalence of mixed-species infections lacked general patterns was based on an analysis of these 15 studies and 22 more conducted between 1929 and 1983, a data set more nearly balanced between these 2 sets of 3 sympatric species. Though the conclusions of these authors encompass many differences in spatial, temporal, and other factors, our results suggest that some of their differences may be attributed to the different sets of species considered in their analyses.

The Garki project in particular pointed to a hierarchy of dynamic relationships implicit in the strong positive associations between *P. falciparum* and *P. malariae* in human individuals, strong seasonal alternation between these species in the human population, and declining prevalences of mixed-species infections with age. In explaining their own conclusions, however, each of our predecessors invoked some unifying concept(s) of heterologous, cross-species immunity.

Immunological and clinical evidence related to cross-species immunity remains scarce, and it implies that major differences as well as similarities probably exist with respect to each shared mechanism. Tumor necrosis factor (TNF), a cytokine, has been strongly implicated in the pathogenesis of cerebral malaria. At a given parasitemia *P. vivax* stimulates greater TNF production than does *P. falciparum* (Butcher et al., 1990; Karunaweera et al., 1992), though cross-reactive antisera can be produced against the TNF-inducing exoantigens of the 2 species (Bate et al., 1992). Carvalho et al. (1997) have demonstrated cross-reactive cellular responses against the *P. falciparum* and *P. vivax* circumsporozoite proteins (CSP), a prominent vaccine target. Anti-CSP antibody densities increase with age in immune responses to *P. vivax* but not *P. falciparum* (Burkot et al., 1989), whereas T-cell responses to *P. falciparum* CSP are typically

short-lived and to *P. vivax* CSP life-long (Zevering et al., 1994). Other shared *P. falciparum*–*P. malariae* (Knowles and Davidson, 1984; Sulzer et al., 1988, 1995), *P. falciparum*–*P. vivax* (Diggs and Sadun, 1965; Kumar et al., 1992; Barnwell and Galinski, 1995), and *P. ovale*–*P. vivax* (Andrysiak et al., 1986) antigens have been reported. With respect to cellular responses, comparative studies have reported that both T-cell density (Merino et al., 1986) and natural killer-cell activity (Saxena et al., 1988) decline in *P. vivax*, but not *P. falciparum*, infections.

The anomalous effects of some interventions may also reflect some aspect(s) of heterologous immunity. Immunoglobulin transfers from West African adults led to the rapid disappearance of *P. falciparum* and the emergence of undetected *P. vivax* infections in 2 Thai adults (Sabchareon et al., 1991) and, in a dually patent West African child, to a relatively slow decline in *P. falciparum*, followed by a still slower decline in *P. malariae* (Cohen et al., 1961). Trials of the SPf66 anti-*P. falciparum* vaccine in Venezuela showed that *P. vivax* incidence increased as *P. falciparum* incidence decreased after vaccinations (Noya et al., 1994). In Gambia, the clinical incidence of *P. malariae* increased, that of *P. ovale* decreased, and *P. falciparum* was unaltered following SPf66 vaccinations (D'Alessandro et al., 1995). In Thailand, the proportion of mixed *P. falciparum*–*P. vivax* infections among positives increased 5-fold following SPf66 vaccination (Luxemburger et al., 1996; Nosten et al., 1997).

Black et al. (1994) have suggested that *P. malariae* infections ameliorate the fevers of subsequent *P. falciparum* superinfections in West African children. A recent field study in the southwest Pacific (Maitland et al., 1996, 1997; Williams et al., 1996) has proposed that *P. vivax* infection reduces the severity of subsequent *P. falciparum* infection, and a study in southeast Asia (Luxemburger et al., 1997) proposes that *P. vivax* attenuates *P. falciparum* severity in dual infections. In dual *P. falciparum*–*P. vivax* infections in neurosyphilis patients, with sequential inoculations the first species inoculated dominated the initial parasitemia, but with simultaneous inoculation, *P. falciparum* always dominated, and with either procedure remained at detectable levels much longer than in single-species infections (Boyd and Kitchen, 1937, 1938; Boyd et al., 1937, 1938; Shute, 1946, 1951). Jeffery (1966) found that *P. falciparum* inoculation following *P. malariae* or *P. vivax* infections produced *P. falciparum* parasitemias higher than those in single-species infections, but *P. falciparum* parasitemias were lower when *P. falciparum* inoculation followed a *P. ovale* infection, as they were following homologous or heterologous *P. falciparum* infections.

In sum, it seems clear that some mechanisms of heterologous, cross-species immunity exist, but their significance awaits further investigation. As before, we hope that our analysis will encourage detailed prospective studies of multispecies phenomena in malaria, with proper concern for the potentially confounding methodological variables. For instance all detection methods are to some degree mediated by densities and durations of infections in peripheral circulation, but, in the right hands, sensitive molecular-level techniques should render this more a lesson in parasite population biology than a problem. Log-linear models may prove useful in such studies. When applied to the data of Chamone et al. (1990), for instance, log-linear models simply confirm the existence of a 3-factor interaction among helminth coinfections in schistosomiasis, but with the data of Willett (1972), they can extend his result to conclude that *Trypanosoma congolense* is fully independent in calves, becoming enmeshed with *Trypanosoma brucei*–*Trypanosoma vivax* interactions only in adult cattle.

We remain most intrigued with the possibility that immune-mediated species interactions influence transmission, perhaps by influencing the relative timing, proportion, or infectivity of gametocytes or sporozoites rather than their abundance per se. Along these lines, a *P. falciparum*–*P. malariae*–*P. ovale* study cited here (Cornu et al., 1986) reported gametocytes of more than 1 species present in 11% of gametocytemic individuals; it would be helpful to

know if this is a typical figure, and if it differs from *P. falciparum*–*P. malariae*–*P. vivax*. Similarly, the Garki project reported that the surplus of mixed-species infections was exaggerated in insecticide-sprayed villages, but the deficit of mixed-species infections in the most closely analogous recent *P. falciparum*–*P. vivax* report (Singh et al., 1989) shows no such pattern. If one considers that the relative importance of durations and intensities of infection, gametocyte production and immune response may vary with the conditions of transmission, it is precisely this sort of information that should lead to useful comparisons of variation within to variation between the species.

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Figure 1.

Relationships between overall prevalence of infection (horizontal axis) and frequency (vertical axis) of tables that show significant (solid bars) or nonsignificant (hollow bars) differences in mixed-species infections from the expected values for a) *P. falciparum*–*P. malariae*–*P. ovale* and b) *P. falciparum*–*P. malariae*–*P. vivax*. The numbers on the horizontal axis represent midpoints of percentage prevalence intervals, e.g., 14.5 represents the interval 10–19%; numbers on the vertical axis represent percentages of the total number of tables within the relevant group (significant or nonsignificant).

Figure 2.

Relationships between overall prevalence of infection (horizontal axis) and frequency (vertical axis) of tables that show significant (solid bars) or nonsignificant (hollow bars) differences in mixed-species infections from the expected values for the 1911–1929 3-species tables (see text). Conventions are as in Figure 1.

Figure 3.

In *P. falciparum*-*P. malariae*-*P. ovale* tables, observed frequencies in single-species cells were generally lower and in dual-species cells generally higher than those expected under the hypothesis of complete species independence, while the reverse was typical of *P. falciparum*-*P. malariae*-*P. vivax* tables. The shared *P. falciparum*-*P. malariae* pair illustrates most of this pattern. In part a, the horizontal axis indicates each of the single-species or dual-species cells, with abbreviations as in the tables; the vertical axis represents frequencies with which observed cell values fell below the values expected under complete independence, for the modern *P. falciparum*-*P. malariae*-*P. ovale* (solid bars), modern *P. falciparum*-*P. malariae*-*P. vivax* (hollow bars) and historical *P. falciparum*-*P. malariae*-*P. vivax* (hatched bars) tables. Part b

shows ratios of observed to expected values in the single-species *P. falciparum* (F) and *P. malariae* (M) cells and the dual *P. falciparum*–*P. malariae* (FM) cell, as a mean value $\pm$ 1 standard deviation, in the significant modern *P. falciparum*–*P. malariae*–*P. ovale* (F, M, O) and *P. falciparum*–*P. malariae*–*P. vivax* (F, M, V) tables.

Figure 4.

Data from human populations with 2, 3, or 4 sympatric *Plasmodium* species imply that neither the overall prevalence of malaria nor the fraction of infections that are mixed-species relate simply to the number of species present. The horizontal axes indicate the 2-species, 3-species, and 4-species data sets, with abbreviations as before (and F, M, X for the 1911–1929 data set), and again a mean value ± 1 standard deviation. In part a, the vertical axis indicates the overall malaria prevalence, and in part b, the vertical axis indicates the ratio of mixed-species to total infections. References for the 2-species data are in McKenzie and Bossert (1997a) for the 3-species data are in Table I and for the 4-species data are Blanchy et al. (1990), Lepers et al. (1990), Denys and Isautier (1991), Anthony et al. (1992), McLaughlin et al. (1993), and Seesod et al. (1997).

Table I

Data from 29 contingency tables.*

Species	LVL	SAMP	PREV	SIG	Reference
F, M, O	A	707	37		Ghary et al., 1988
		225	40		Deloron et al., 1989
		253	50		Deloron et al., 1989
		1,048	55		Landgraf et al., 1994
		222	65		Deloron et al., 1989
		196	79		Campbell et al., 1987
		163	80		Hellgren et al., 1994
		210	82		Mwanakasale et al., 1995
		142	82		Campbell et al., 1987
		245	47	S	Deloron et al., 1989
		147	48	S	Campbell et al., 1987
		1,465	47		Thomson et al., 1994
		475	57		Deloron et al., 1989
		742	23	S	Cornu et al., 1986
		470	43	S	Deloron et al., 1989
		485	71	S	Campbell et al., 1987
		8,539	74	S	Trape et al., 1994
		2,465	4		Trape et al., 1992
		2,192	31		Wilding et al., 1995
F, M, V	A	15,155	53	S	Spencer et al., 1987
		340	14		Yadav et al., 1991
		234	23		Yadav et al., 1991
		206	40		Graves et al., 1989
		574	17		Yadav et al., 1991
		268	22		Gordon et al., 1991
		3,462	23	S	Beljaev et al., 1987
		614	34	S	Collins et al., 1988
		913	47	S	Banchongaksorn et al., 1996
		506	54	S	Mizushima et al., 1994
	B				
	D				

* Abbreviations: level of analysis, LVL; sample size, SAMP; overall prevalence, PREV (%); and statistical significance, SIG, where S indicates a table in which the observed prevalences differ from those expected (see text); F, M, O, V indicate occurrences of *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*.

Table II

Summary of 11 significant tables.*

Species	LVL	F, M/X	F, X/M	M, X/F	Reference
F, M, O	A	S			Deloron et al., 1989
		S			Campbell et al., 1987
	B	S	S		Deloron et al., 1989
		S			Campbell et al., 1987
F, M, V	C	S	S	S	Cornu et al., 1986
	D	S	S	S	Trape et al., 1994
		S	S	S	Spencer et al., 1987
	D	S	S		Beljaev et al., 1987
		S	S		Collins et al., 1988
			S		Banchongaksorn et al., 1996
				S	Mizushima et al., 1994

* Abbreviations: level of analysis, LVL; F, M/X indicates *P. falciparum* and *P. malariae* given X, where X represents either *P. ovale* or *P. vivax*; F, X/M and M, X/F correspond; S indicates a table in which observed prevalences differ from those expected (see text and Table I).