Published in final edited form as:
J Med Entomol. 1997 July ; 34(4): 417–425.

Mixed-Species *Plasmodium* Infections of *Anopheles* (Diptera: Culicidae)

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Abstract

Mixed-pathogen infections of vectors rarely are considered in the epidemiological literature, although they may occur in nature. A review of published reports shows that many *Anopheles* species are capable of carrying sporozoites of > 1 *plasmodium* species, of doing so simultaneously in field conditions, and of acquiring and transmitting these in experimental situations. Mixed-species infections in mosquito populations occur at frequencies greater than or equal to the product of the constituent species prevalences, whereas human populations have apparent mixed-species infections at frequencies less than or equal to their corresponding expected values. We present a model for the accumulation of parasite infections over the lifespan of a mosquito that explains this surplus of mixed-species infections. However, the expected frequencies of mixed infections on the basis of our model are greater than those found in nature, indicating that the sampling by mosquitoes of *Plasmodium* species from human malaria infections may not be random.

Keywords

Anopheles spp; *Plasmodium* spp; malaria transmission; parasite ecology

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The Pioneering studies of parasite community dynamics have focused on helminths (e.g., Schad 1963, Kennedy 1975, Holmes 1983, Esch et al. 1990). Recently Dobson (1985, 1990) and Dobson and Roberts (1994) have stressed the role of species life-histories in structuring helminth communities and have extended the general ecological principle that as a constituent species becomes more aggregated in its distribution, the relative importance of interspecific competition declines in its population regulation. Although data on the aggregation of *Plasmodium* species in *Anopheles* is essentially anecdotal, its variability indicates that *Plasmodium* species interactions may warrant more concerted investigation.

In mixed-species malaria infections of humans, one of the constituent *Plasmodium* species typically dominates (e.g., Mayne and Young 1938, Molineaux and Grammicia 1980, Looareesuwan et al. 1987, Fox and Strickland 1989). Cohen (1973) analyzed the epidemiological literature and reported a general deficit of detectable mixed-species infections, associated this deficit with splenomegaly, and inferred an underlying heterologous immunity. Hence, the excess of mixed-species infections found during the Carlo project was unexpected, but it also was interpreted as representing species interactions of predominantly cooperative (Cohen and Singer 1979) or predominantly competitive (Molineaux and Grammicia 1980) character. Richie (1988) emphasized competitive interactions in an evolutionary context, suggesting that co-infections promote antigenic divergence, but also proposed a role for mutual facilitation in the ecological succession of *Plasmodium* species within a mammalian host.

Neither the interactions of pathogen species within vectors or the pathogen sampling processes embodied in human-vector contacts have received much attention in the infectious-disease

literature. Most mathematical models of parasite-host interactions (e.g., Cohen and Singer 1979, Beck 1984, Bremermann and Thieme 1989, Anderson and May 1991) exclude the possibility that several pathogen species or strains can be transmitted (or cleared) simultaneously. Empirical studies show that in some *Aedes* species, coinfecting microfilariae can suppress *P. gallinaceum* Brumpt development (Albuquerque and Ham 1995), whereas coinfecting microfilariae or *P. gallinaceum* enhance arbovirus transmission (Turell et al. 1984, Paulson et al. 1992, Vaughan and Turell 1996). It also has been proposed that microfilariae and *Plasmodium* species retard the development of each other in *Anopheles gambiae* Giles s.l. (Muirhead-Thomson 1953), and that the high frequency of such coinfections in *An. punctulatus* Doenitz is balanced epidemiologically by increased mosquito mortality (Burkot et al. 1990).

If mixed-species *Plasmodium* infections of *Anopheles* have similar (or any other) effects, these and their epidemiological consequences apparently remain unexplored. In this article, we assemble evidence that many *Anopheles* species can carry and transmit >1 *Plasmodium* species simultaneously. The sparse data available indicate remarkable variability in the relationships of mixed-species *Plasmodium* prevalence in *Anopheles* to mixed-species prevalence in corresponding human populations and the prevalence expected under the hypothesis of species independence.

One important characteristic of the process by which mosquitoes sample pathogens is that they sample repeatedly over time, for instance every other day. Because vectors apparently do not clear pathogens once infected, the sampled pathogens accumulate over the lifespan of the mosquito. Therefore, we would expect that older mosquitoes would exhibit a higher frequency of multiple infections than either the prevalence of multiple infections in humans or the product of the prevalences of the individual species, the usual null statistical hypothesis. Younger mosquitoes would have a lower prevalence of multiple infections than older ones, so the overall prevalence of multiple infections in vectors depends on the lifespan and age distribution of the vector population. We present a simple model of the sampling and accumulation process that gives an indication of the degree to which the prevalence of multiple infections in the vector population might be increased.

Materials and Methods

The detection of mixed-species mosquito infections through microscopy is not possible (e.g., Shute and Maryon 1952), but it may be accomplished using enzyme immunoassays. We located our sources by consulting standard references (e.g., Wernsdorfer and McGregor 1988) and surveying recent English- and French-language journals in the Countway and Mayr Libraries at Harvard University. We used the *G*-test with the Williams correction (Sokal and Rohlf 1981) to analyze contingency tables of prevalence data.

For simplicity, in constructing a heuristic model we assumed that P_A and P_B, the prevalences of infectious gametocytes for 2 *Plasmodium* species in the human population, are constant (i.e., that the parasite populations in the human and mosquito populations are in joint equilibrium or are not dynamically linked). Other parameters we consider are the 2-d mosquito mortality (s), and duration of sporogonic cycle (D), Our units of time (t), correspond to an idealized 2-d gonotrophic cycle, typical for many tropical species in the subgenus *Cellia.*

Given the further assumptions that mosquitoes bite only humans and efficiently acquire infections from infectious gametocyte carriers, we note that $(1 - P_j)^t$ represents the probability that at age t a mosquito has not been infected with parasite species j, and $1 - (1 - P_i)^t$ represents the probability that at age t a mosquito has been infected at least once with species i. We assume

constant, continuous mortality in the mosquito, so e^{-sk} represents the probability that a mosquito survives to age k.

We then express the probabilities that a mosquito of age $D + t$ is infectious (no mosquitoes younger than $D + 1$ can be infectious) and sum over t and divide by the total number of mosquitoes to obtain the frequencies of mosquitoes that are infectious:

for species A and only species A, as

$$V_A = \left\{ \sum_{t=1}^{\infty} [1 - (1 - P_A)^t](1 - P_B)^t e^{-s(D+t)} \right\} \div \sum_{t=1}^{\infty} e^{-st}, \quad (1)$$

for species B and only species B, as

$$V_B = \left\{ \sum_{t=1}^{\infty} [1 - (1 - P_B)^t](1 - P_A)^t e^{-s(D+t)} \right\} \div \sum_{t=1}^{\infty} e^{-st}, \quad (2)$$

for neither species A nor species B, as

$$V_0 = \left\{ \sum_{t=1}^{\infty} [(1 - P_A)(1 - P_B)]^t e^{-s(D+t)} + \sum_{t=1}^{\infty} e^{-si} \right\} \div \sum_{t=1}^{\infty} e^{-st}. \quad (3)$$

Therefore, the prevalence of mosquitoes simultaneously infectious for both species A and species B, is

$$V_{AB} = 1 - (V_A + V_B + V_0). \quad (4)$$

The fraction of positives that is multiply infectious is $V_{AB}/(1 - V_0)$.

We translated this model into a BASIC program to approximate and graph numerical solutions. The summations were truncated at $t = 50$ (100 d), because for the survivorship values of interest the probability of a mosquito surviving beyond this age is remote.

Results

At least 39 species of *Anopheles* are known to be capable of transmitting > 1 species of *Plasmodium* (Table 1). Individuals of at least 7 of these species have been detected carrying sporozoites of > 1 *Plasmodium* species in the field, and individuals of an additional 4 species simultaneously have transmitted 2 species in experimental settings.

With few exceptions, the available frequency information about mixed *Plasmodium* species in the 7 *Anopheles* species applies only to positives (i.e., we have data on multiple- and single-species infections but not on overall prevalence). In 2 studies the frequency of mixed-species infections among mosquito positives was less than that among human positives, and in another 3 the converse was true (Table 2). Data from the remaining studies indicated that different circumstances in the same location might lead to either of these relationships, as might analyses conducted at different temporal and spatial scales.

The comparative figure for humans in Kenya, as cited by Beier et al. (1988), refers to a 1980–1983 study of 36 villages in the Saradidi region (Spencer et al. 1987); the entomological study by Beier et al. (1988) took place in 1986 in 2 of these villages, whereas that of Beier et al. (1991) took place in 1987–1988 in the same villages. The 2 complete sets of Thai figures (Rosenberg et al. 1990a, b) apply to the 1st and 2nd yr of a village study; in each year the prevalence of mixed-species infections in mosquitoes fit the hypothesis of the statistical independence of the species, whereas that in humans was less than half the expected value. The 3rd Thai figure (Gingrich et al. 1990; for mosquitoes only) applies to the following 2 yr in the same village; the observed mixed-species prevalence in mosquitoes again fit the product of the singles species prevalences.

The entomological studies conducted by Burkot et al. (1990, 1992) in Buksak village, Papua New Guinea, implied that the fraction of positive *An. punctulatus* co-infected with *P. falciparum* and *P. vivax* peaked during early 1987, shirting from 6% for 1986 to 8–9% for January 1986–March 1987 to 1% for 1987. The comparative figures for humans (from Burkot et al. 1987) apply to a 1983–1985 study of 8 other villages near Madang, and are far higher than the 1–4% found during 1981–1983 surveys of 53 villages in this coastal province (Cattani et al. 1986). In Buksak, for 1986 and 1986–1987 the observed prevalence of mixed-species infections in resting catches (6 and 9% of positives, respectively) far exceeded expected values, whereas the observed prevalence in 1987 resting catches and 1986–1987 biting catches (1 and 8% of positives, respectively) fit expected values. We were not able to derive sufficiently detailed comparable figures for humans. In the other New Guinea study, from the highlands of Irian Jaya (Anthony et al. 1992), mixed-species prevalence in mosquitoes fit the expected values; again we could not extract complete, exact comparative figures for humans from this report, but we strongly suspect that the human data show a substantial deficit of mixed-species infections.

Figures 1 and 2 illustrate behaviors of our model, with a fixed 14-d incubation period (i.e., $D = 7$) and, for simplicity, $P_A = P_B$. Figure 1 sets P_A (= P_B) = 0.05 or 0.20, an idealized prevalence of infectious gametocytes in the human population (i.e., $P_A + P_B - P_AP_B$) of 0.0975 or 0.36, respectively. As one would expect, the infectious fraction of the mosquito population and the ratio of dually to singly infectious vectors decline with increasing vector mortality. Notice, however, that in every case the frequency of multiple infections is much larger than P_AP_B and much larger than observed.

Figure 2 fixes the vector mortality (s), at 0.28. The corresponding 0.85 daily survivorship is within published ranges for *An. gambiae* in East Africa, albeit near the upper end of recent ranges (e.g., Macdonald 1956,Garrett-Jones and Shidrawi 1969,Mutero and Birley 1987,Gillies 1988). The frequency of multiply infectious mosquitoes increases with increasing prevalence in humans, while the frequency of singly infectious mosquitoes attains a maximum at an intermediate value of P. Hence, with increasing P the ratio of dually to singly infectious mosquitoes increases at a slightly greater than linear rate.

Figure 3 compares the frequencies of multiply infectious mosquitoes predicted by our model to those predicted by the product of species prevalences in infectious mosquitoes and addresses the typical case in which the gametocyte prevalences P_A and P_B are not equal.

Discussion

The information compiled here indicates that many *Anopheles* species are capable of carrying sporozoites of >1 *Plasmodium* species, of doing so simultaneously under field conditions, and of acquiring and transmitting these in experimental situations. The data available to us indicate that there is wide variability in mixed-infection frequencies in humans and mosquitoes, in both

absolute and relative terms, and that this variability may be related to variability in temporal, spatial, methodological or other factors. For example, we were not able to assess the effects of differing *Plasmodium* species composition (e.g., the potential presence of all 4 human *Plasmodium* species in New Guinea, or the substitution of *P. ovale* for *P. vivax* in most of Africa) or subdivisions within *Anopheles* species complexes. Although the sheer number and complex interconnections of such variables seem overwhelming, it may be that neither these nor many other phenomena of malaria demand detailed local explanation.

In contrast to the situation in human populations, the few data points for which absolute prevalences are available do not indicate prevalences of mixed-species infections in mosquito populations less than those expected on the basis of multiplying single-species prevalences. There is no difficulty explaining this phenomenon on the basis of the simple accumulation model presented here. What may require further explanation is that the prevalences of mixed-species infections do not reach the levels predicted by our model, with the exception of the single positive *An. fluviatilis* collected by Subbarao et al. (1992). A far more complex model will incorporate the possibilities that humans also accumulate infections, mosquitoes may feed on other mammals, and infectious humans may fail to infect mosquitoes. However, it appears unlikely that the net bias introduced by our simplifying assumptions would account for order-of-magnitude discrepancies. Perhaps the distinction is mediated by both relative gametocyte prevalence (Burkot et. al. 1987, Rosenberg et al. 1990b) and infectivity. Graves et al. (1988) noted that the presence of *P. falciparum* gametocytes appeared to reduce the infectivity of *P. vivax* gametocytes, but not of *P. malariae* gametocytes, when either was present simultaneously. We hope that future studies will address these points, the accumulation processes by which such patterns may arise, and the potential epidemiological effects (e.g., in situations in which most mixed-species infections in mosquitoes are derived from blood-meals from several different humans [Davies 1990, Conway and McBride 1991, Klowden and Briegel 1994] rather than from meals on multiply infected humans).

There are many pitfalls in the diagnosis of mixed-species infections and few precedents for the study of such infections in concurrent human and mosquito populations. However, the utility of such investigations could well reach beyond *Plasmodium*, *Anopheles*, or interactions solely at the species level. Recent studies have found *Borrelia* and *Babesia* co-infections in individual ticks (Mather et al. 1990), dual *Leishmania* species infections in single sandflies (Barrios et al. 1994) and mixtures of trypanosome genotypes in individual *Glossina* (Letch 1984, Stevens et al. 1994). Although the frequencies and implications of recombination among *Plasmodium* genotypes prompt debate (Tibayrenc et al. 1990, Walliker 1991, Read and Day 1992, Ranford-Cartwright et al. 1993, Babiker et al. 1994, Paul et al. 1995), there is no debate that if *Plasmodium* genotypes are to recombine they must co-occur as gametes in the same mosquito. It is curious that there has been little discussion of circumstances or phenotypic characteristics that might facilitate or hinder this process. Here, we suggest that at least at the species level, prospective interactions are not likely to be bounded by one-at-a-time transmission, hence phenomena that might be considered competitive or cooperative may be even more important than previously suspected. It is not clear whether the consequences of incorporating simultaneous acquisitions or losses of multiple infecting species in human epidemiological models (e.g., Cohen and Singer 1979) would be trivial or profound, but 2 recent models (May and Nowak 1995, van Baalen and Sabelis 1995) suggest the latter view.

Acknowledgements

We gratefully acknowledge the contributions of S. Austad, M. L. Bossert, R. Didday, P. Fischer, E. Kane, R. Schwabacher, A. Spielman, T. Tsomides, 2 anonymous reviewers, and the Countway and Mayr libraries at Harvard University. Some of this material is based on preparatory work supported under a National Science Foundation Graduate Fellowship awarded to F.E.M. We appreciate the continued support of the Maurice Pechet Foundation.

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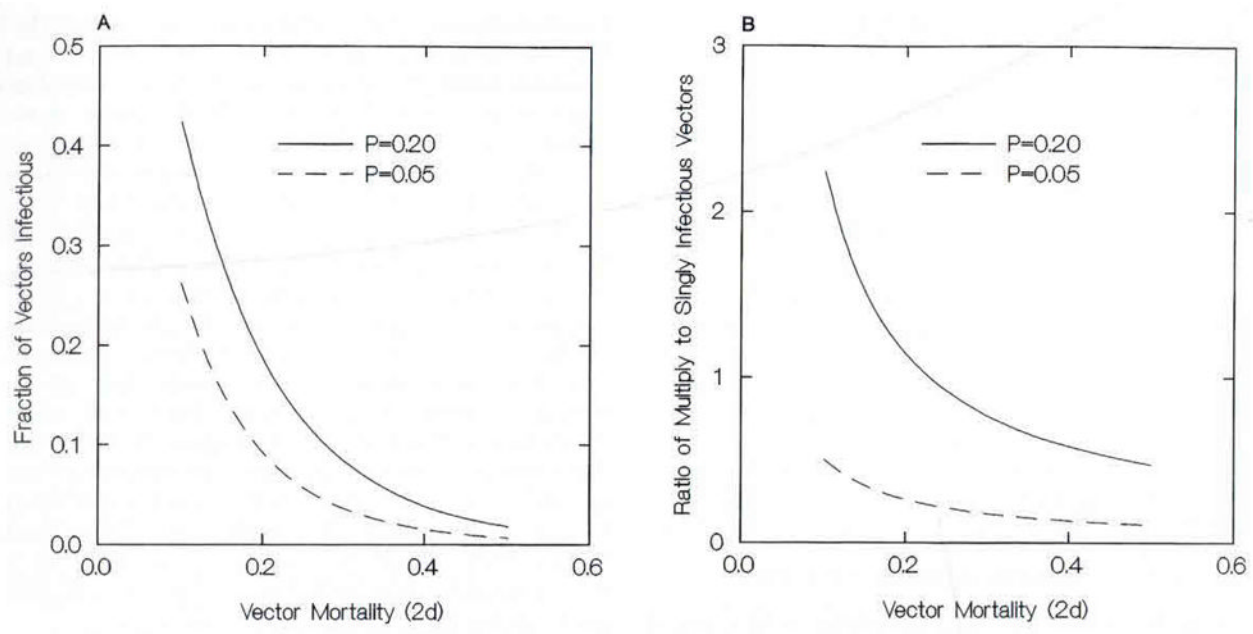

Fig. 1.

Behavior of the accumulation model for 2 values of the species gametocyte prevalences in the human population, $P = P_A = P_B$, with respect to 2-d vector mortality: (A) the fraction of vectors infectious for any species or combination; (B) the ratio of vectors infectious for >1 species to those infectious for only 1.

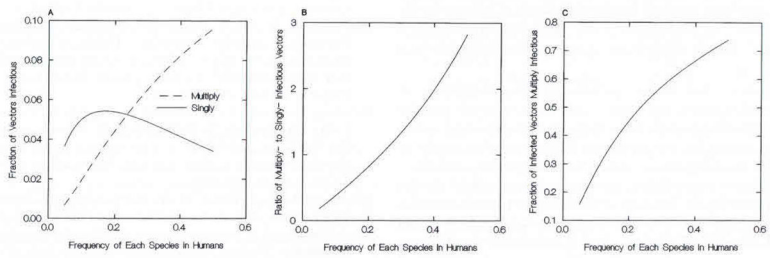

Fig. 2.

Behavior of the accumulation model, for 2-d vector mortality fixed at 0.28, with respect to the species gametocyte prevalences in the human population, $P = P_A = P_B$: (A) the fraction of vectors infectious either for a single species alone or for >1; (B) the ratio of vectors infectious for >1 species to those infectious for only 1; (C) the ratio of vectors infectious for >1 species to the total infectious.

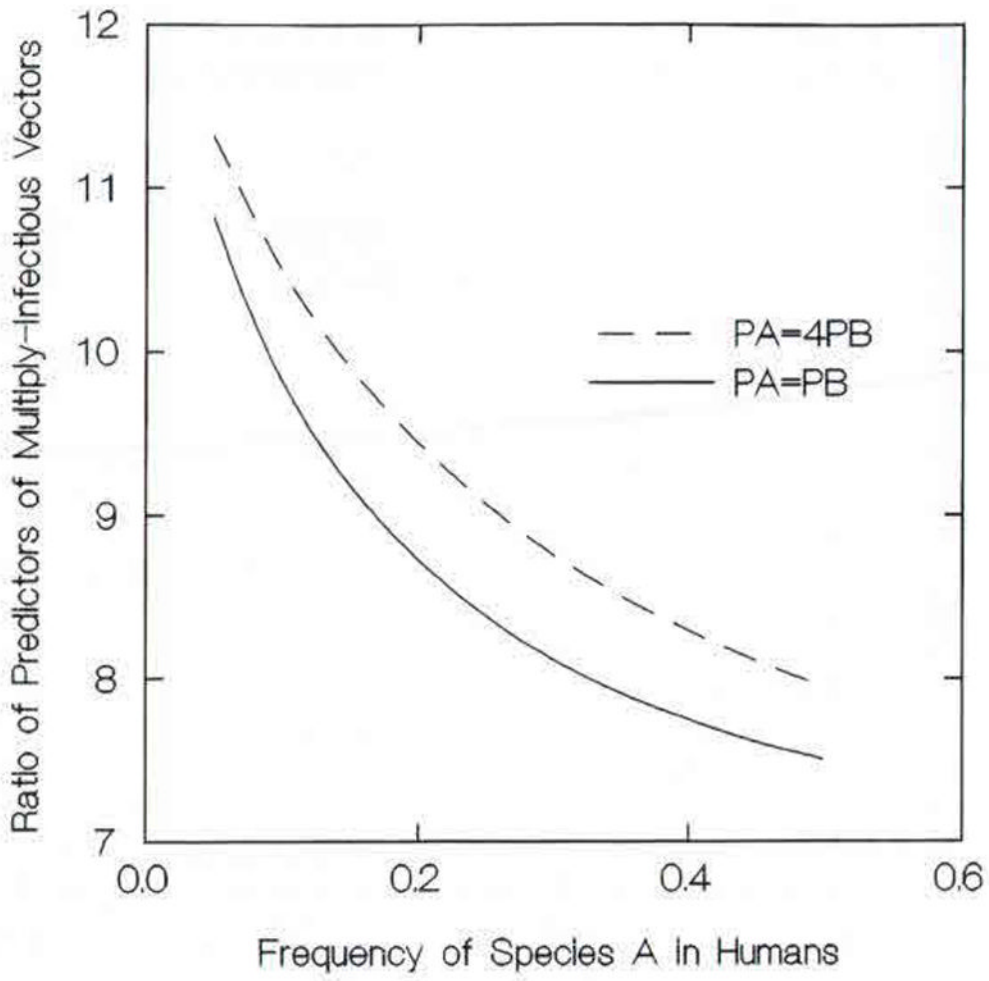

Fig. 3.

Behavior of the accumulation model, for 2-d vector mortality fixed at 0.28, with respect to equal and unequal species gametocyte prevalences in the human population, P_A and P_B. The ratio shown divides the frequencies of multiply infectious mosquitoes predicted by the model by those predicted by the product of the species prevalences in infectious mosquitoes.

Table 1

Anopheles species known to be capable of transmitting >1 species of Plasmodium, singly or concurrently

Anopheles	Single-species infections					Mixed-species infections	
	FAL	MAL	OVA	VIV	Refs	Refs (Field)	Refs (EXP)
An. albimanus Wiedemann	x	x		x	Beach et al. 1992, Molineaux 1988, Olano et al. 1985		
An. amictus Edwards	x			x	Molineaux 1988		
An. annularis van der Wulp	x	x		x	Amerasinghe et al. 1991		
An. annulipes Walker	x			x	Molineaux 1988		
An. atroparvus Van Thiel	x	x	x	x	Molineaux 1988		28 FV
An. balbacensis Baisis s.l.	x	x	x		Molineaux 1988		
An. bancrofti Giles	x			x	Molineaux 1988		
An. culifacies Giles s.l.	x			x	Amerasinghe et al. 1991, Mendis et al. 1990	29 FV	
An. darlingi Root	x			x	Klein et al. 1991a,b		
An. deaneorum Rosa-Freitas	x			x	Branquinho et al. 1993		
An. dirus Peyton and Harrison	x			x	Baker et al. 1987, Gingrich et al. 1990, Harbach et al. 1987	27 FV	
An. farauti Laveran	x	x		x	Burkot et al. 1987, Wirtz et al. 1987		19 FM/FV
An. fluviatilis James	x			x		29 FV	
An. freeborni Aitken	x	x	x		Molineaux 1988		
An. funestus Giles	x	x	x			5, 6, 30 FM/FO/MO/FMO	
An. gambiae Giles s.l.	x	x	x	x	Collins & Roberts 1991	5, 6, 15, 30 FM/FO/MO/FMO	
An. koliensis Owen	x			x	Burkot et al. 1987, Wirtz et al. 1987		
An. lindesayi pleccau Koidzumi	x	x		x	Lien 1991		
An. longirostis Brug	x			x	Molineaux 1988		
An. ludlowae Theobald	x	x		x	Lien 1991		
An. maculatus Theobald	x	x	x	x	Lien 1991, Molineaux 1988	18 (F, M, V "mixed")	
An. mediopunctatus Theobald	x			x	Klein et al. 1991a,b		
An. minimus Theobald	x	x		x	Gingrich et al. 1990, Harbach et al. 1987		
An. nigerrimus Giles	x			x	Baker et al. 1987		
An. nili Theobald	x	x			Boudin et al. 1991		
An. oswaldoi Peryassu	x	x		x	Branquinho et al. 1993, de Arruda et al. 1986		
An. peditaeniatus Leicester	x			x	Baker et al. 1987		
An. punctipennis Say	x	x		x	Molineaux 1988		
An. punctulatus Doenitz	x	x		x	Burkot et al. 1987, Wirtz et al. 1987	2, 11, 12 FV	
An. quadrimaculatus Say	x	x	x	x	Molineaux 1988		8 FV
An. sacharovi Favre	x			x	Molineaux 1988		
An. sinensis Wiedemann	x	x		x	Lien 1991		
An. splendidus Koidzumi	x	x		x	Lien 1991		
An stephensi Liston	x	x	x	x	Molineaux 1988		26 FV
An. stipmaticus Skuse	x			x	Molineaux 1988		
An. subpictus Grassi	x	x		x	Amerasinghe et al. 1991		
An. tessellatus Theobald	x	x		x	Gamage-Mendis et al. 1993, Lein 1991		
An. triannulatus Neiva and Pinto	x			x	Klein et al. 1991a,b		
An. vagus Donitz	x			x	Baker et al. 1987		

Refs, references; FAL or F, *P. falciparum;* MAL or M, *P. malariae;* OVA or O, *P. ovale;* VIV or V, *P. vivax;* FV, FM, FO, MO, or FMO, species in mixed infections; for field or experimental (EXP) studies. Beach et al. 1992; Collins & Roberts 1991; Klein et al, 1991a,b; Lien 1991; Olano et al, 1985 (for single-species infections) also involve experimental rather than field studies. Molineaux 1988 reviews microscopy-based studies. Boyd et al. 1937, Collins & Roberts 1991, Gamage-Mendis et al. 1993, Graves et al. 1988, Klein et al. 1991a,b, Lien 1991, Olano et al. 1985, Shute 1951 report

results from microscopy; Amerasinghe et al. 1991, Anthony et al. 1992, Baker et al. 1987, Branquinho et al. 1993, Burkot et al. 1990, Burkot et al. 1992, Fontenille et al. 1992, Harbach et al. 1987. Mendis et al. 1990, Subbarao et al, 1992 from immunoassay; Beach et al. 1992, J. Beier et al. 1991, M. Beier et al 1988, Boudin et al. 1991, Burkot et al, 1987, de Arruda et al, 1986. Gingrich et al. 1990, Gordon et al. 1991, Ponnudurai et al. 1990, Rosenberg et al, 1990, Trape et al. 1994, Wirtz et al. 1987 from both. In nineteen of twenty references microscopy revealed salivary glands positive for sporozoites, but in de Arruda et al. 1986 only midguts positive for oocysts.

Table 2

Frequencies of mixed Plasmodium species in *Anopheles* species and in corresponding human populations

Location	Plasmodium	*Anopheles*	Mixed as % of positives		Refs
			Mosquitoes	Humans	
Senegal	F, M, O	*An. gambiae*	12	30	Trape et al. 1994
		An. funestus	17		
Kenya	F, M, O	*An. gambiae*	16	11	M. Beier et al. 1988, Spencer et al. 1987
		An. funestus (both spp.)	14		
			3		J. Beier et al. 1991
Madagascar	F, M, O, V	*An. gambiae*	2	38	Fontenille et al. 1992
India	F, V	*An. culifacies*	33	3	Subbarao et al. 1992
		An. fluviatilis	100		
Thailand	F, V	*An. dirus*	5	4	Rosenberg et al. 1990a,b
			0	5	
			4		Gingrich et al. 1990
Malaysia	F, M, V	*An. maculatus*	44	36	Gordon et al. 1991
New Guinea	F. M, O, V	*An. punctulatus*	11 (FV)	3 (2FV)	Anthony et al. 1992
	F, M, V		1–9 (FV)	4–16 (FV)	Burkot et al. 1987, 1990, 1992

Abbreviations as in Table 1.