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MIXED-SPECIES *PLASMODIUM* INFECTIONS OF HUMANS

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Abstract

We analyzed point-prevalence data from 35 recent studies of human populations in which *Plasmodium falciparum* and one other *Plasmodium* species were the reported causes of malaria infections. For the *P. falciparum*–*Plasmodium vivax* pair, higher overall prevalence in a human population is associated with fewer mixed-species infections than expected on the basis of the product of individual species prevalences. This is not true for *P. falciparum*–*Plasmodium malariae*.

Our current understanding of parasite communities rests on several decades of fruitful studies of helminths (Noble, 1960; Schad, 1963; Kennedy, 1975; Scott and Gibbs, 1986; Stock and Holmes, 1988; Esch et al., 1990; Fernandez and Esch, 1991; Booth and Bundy, 1992; Kennedy and Bush, 1992). Many of the ecological and evolutionary insights from these pioneering studies could be tested among parasitic protists as well, building from analyses of relatively static, simple patterns among a few congeners toward those of dynamic interactions among multiple genera. Among helminths, for instance, Dobson (1985, 1990; Dobson and Roberts, 1994) has emphasized that aggregated intraspecific distributions may diminish interspecific competition, and Lotz and Font (1991, 1994; Lotz et al., 1995) have stressed that species rarity and recruitment may modulate patterns of interspecific association.

Four species of *Plasmodium* cause human malaria, *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. Sympatric combinations of these species occur in human populations and within infected individuals. Various phenomena associated with species co-occurrence have been studied as such for more than a century, e.g., Thayer and Hewetson (1895), since an era in which the very existence of more than one species was subject to debate. Biogeographic anomalies such as the absence of *P. vivax* and presence of *P. ovale* in West Africa and the spotty worldwide distribution of *P. malariae* have periodically attracted intense interest (see Molineaux, 1988), as have the frequencies of species co-occurrence within individuals and the possible correlates of mixed-species infections. This paper focusses on the latter 2 topics, particularly in those human populations in which recent reports suggest that only *P. falciparum* and 1 other *Plasmodium* species co-occur.

In a massive survey of *Plasmodium* species prevalence in humans, Knowles and White (1930) recorded mixed-species infections at various frequencies at various sites on all 6 inhabited continents and noted that particular species combinations were often seasonal. They complained that mixed-species infections were often underreported, and demonstrated the phenomenon in a test of microscopists. Cohen's (1973) analysis of the epidemiological literature concluded that deficits in the reported prevalence of mixed-species infections were not wholly artifactual, but common, often statistically significant phenomena with a previously unsuspected biological basis in heterologous immunity. His hypothesis was later extended to encompass the seasonal species patterns and surplus prevalence of mixed-species infections detected during the Garki project (Molineaux and Gramiccia, 1980; Molineaux et al., 1980). Richie's analysis (1988) argued that species coexistence was made possible only through antigenic divergence and hence the avoidance of heterologous immunity. He suggested that

competitive interspecific suppression in simultaneous infections might be balanced by some form of reciprocal successional facilitation.

Unfortunately, few recent epidemiological reports examine mixed-species malaria infections; the studies by Fox and Strickland in Pakistan (1989) and Rosenberg et al. in Thailand (1990) are unique in reporting and discussing their findings that mixed *P. falciparum*–*P. vivax* infections occur at the levels expected and at levels far less than expected, respectively. The frequency with which unsuspected *P. vivax* infections emerge when protected patients clear their *P. falciparum* infections, in a variety of locations (Looareesuwan et al., 1987; Takagi et al., 1988; Nguyen and Keystone, 1989; Schuurkamp, 1992), hints that many mixed-species infections still escape detection. Though it is conceivable that this phenomenon derives solely from *P. vivax* hypnozoites, molecular-level detection methods produce wildly disproportionate increases in the reported prevalence of mixed-species infections (Barker et al., 1989; Relf et al., 1990; Brown et al., 1992; Arai et al., 1994) and may do so with several combinations of species; in studies comparing polymerase chain reaction (PCR) techniques to microscopy, for example, Snounou, Pinheiro et al. (1993) found that in Guinea Bissau mixed-species cases accounted for roughly half of a 142% increase and in Thailand (Snounou, Viriyakosol et al., 1993) three-quarters of a 22% increase in infections detected.

Although *P. ovale* was seldom recognized as a distinct etiologic entity until the 1960s, reports of human populations in which just 1 or 2 *Plasmodium* species caused malaria became typical only as the global malaria-eradication campaigns of that era subsided. Accordingly, our predecessors based their analyses almost exclusively on studies in which 3 *Plasmodium* species were reported present. As we hypothesize that some conclusions about mixed-species *Plasmodium* infections may vary with the number or identity of the species involved, or both, we defer our examination of similar polysympatric circumstances and questions of heterologous immunity to a later paper.

Here we address questions of spatial and temporal heterogeneity in the prevalence of dual infections. Cohen (1973) briefly examined geographic heterogeneity and seasonal variation in the contexts of “parasite rate,” i.e., overall prevalence, and “ecology,” respectively, and concluded that both factors (and both contexts) were at most minor influences. Richie (1988) considered both factors, at least implicitly, and concluded that no general relationships could obtain because “nearly every imaginable prevalence pattern has been found by one survey or another ... there are geographic differences in the way in which human malaria species interact and ... these interactions may even change from year to year in a given locale.” In this paper we analyze recent epidemiological data to determine if distinct prevalence patterns exist and, if so, if their influence is as weak as Cohen suggested and as confined to particular points in space and time as Richie proposed.

MATERIALS AND METHODS

We surveyed English- and French-language epidemiological reports published from 1984 to 1995, inclusive, and available in Countway or Mayr libraries at Harvard University; thus, the epidemiological information we used was more recent than that used in Richie’s (1988) or Cohen’s (1973) analyses of mixed-species *Plasmodium* infections. Many recent studies report results only for *P. falciparum* even if other species were also 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, and that in some manner explicitly report the presence or absence of multiple species and of mixed-species infections, and if present explicitly distinguish the numbers and species involved. In 2-species cases, however, we assumed the obvious interpretation of “mixed infections.” We excluded studies that detected only antibodies and those that do not fully

identify the period, place, and methods of sample collection. When the same data appeared in more than 1 eligible report, we analyzed only the more comprehensive set. Given a choice between data gathered in mass surveys and data gathered by other methods in the same community during the same period, we used only the mass-survey data. For the several studies that used molecular-level detection techniques as well as microscopy, we analyzed only the microscopy results.

Our analytical objectives paralleled those of our predecessors, and our analytical methods paralleled those of Cohen (1973) in particular. Our truncated chronological range and restriction to complete, exact data produced several advantages. Because the data were not censored, we could base our comparisons on significance tests with categorical values, rather than on confidence limits on parameter estimates (see Cohen, 1971). We could more readily identify studies in which repeated sampling might have occurred. We could assume that *P. ovale* and *P. vivax* had been properly distinguished. The clear disadvantage of our approach was that the information we compiled was too limited to allow analyses of many variables considered in Richie's verbal, or Cohen's statistical, or both, analyses; for instance, we found little recent data that relate mixed-species infection prevalence to spleen size.

Among studies that report exactly 2 *Plasmodium* species and meet our criteria, only 2 analyze their findings with respect to mixed-species infections (Fox and Strickland, 1989; Rosenberg et al., 1990), so we could not perform a meta-analysis in the literal sense, e.g., Hedges and Olkin (1985); we did not pool or partition studies or their reported results as such, e.g., Friedenreich (1993), except in grouping studies by the number and identities of the *Plasmodium* species involved. Instead, we extracted categorical point-prevalence data from each report to construct contingency tables, each of which contained 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 a single 0 entry in a table, we added 0.5 to each cell to estimate the test statistic (Pagano and Gauvreau, 1993); given 2 zero entries in a table (as occurred in just under 5% of tables; see below), we eliminated it from further analysis. We tested each table against the standard null hypothesis of species sampling independence, i.e., that the dual-infection prevalence is the product of the individual species prevalences. Because none of the marginal totals was fixed, we used the *G*-test, with Williams' correction (Sokal and Rohlf, 1981). Following Cohen (1973), we counted a table for which $P < 0.01$ significantly different from that expected.

Our predecessors examined hypotheses about spatial and temporal heterogeneity at the level of specific sites, seasons, and/or years. We hypothesized further that conclusions about prevalence patterns of mixed-species infections might vary with the particular spatial and temporal scales of analysis. We considered our basic, lowest-level sampling unit (level A) to be a single village sampled during a period lasting no longer than 1 mo, our most-aggregated, top-level sampling unit (level D) to be multiple villages sampled over over a period of multiple months, and the intermediate units to be multiple villages sampled within 1 mo (level B) and/or 1 village sampled over multiple mo (level C). Because species prevalence data from each village each month might constitute a contingency table (albeit usually a tacit one), a single epidemiological report might produce only 1 contingency table, several, or many. Because we had no basis for making assumptions about any underlying distribution(s) of prevalences in the studies, we used the nonparametric Mann–Whitney test for distributional differences (Sokal and Rohlf, 1981).

RESULTS

Table I summarizes general information about *Plasmodium* species occurrence. At the level of human populations, recent authors have detected 8 of the 15 possible species combinations,

including the co-occurrence of all 4 species in populations in Madagascar and New Guinea. Among the missing combinations, *P. malariae* and *P. vivax* were most recently reported to co-occur without *P. falciparum* in a remote region of Peru (Sulzer et al., 1975). The lack of the Duffy antigen/chemokine receptor in most West African populations excludes *P. vivax*. At the level of human individuals, recent authors have detected 11 of the 15 possible species combinations. The 4 missing combinations are those that include the simultaneous presence of *P. ovale* and *P. vivax*. The only reported instance of co-occurrence of all 4 species in an individual relied on PCR-based detection techniques (Snounou, Viriyakosol et al., 1993).

Thirty-five reports fulfill our criteria and report the prevalence of exactly 2 *Plasmodium* species. Seven studies from Africa report the co-occurrence of *P. falciparum* and *P. malariae*, and 2 of *P. falciparum* and *P. ovale*. Nineteen studies from south Asia, 5 from southeast Asia, and 2 from South America report the co-occurrence of *P. falciparum* and *P. vivax*. From these 35 reports, we extracted 219 contingency tables, eliminated 10 that indicated the presence of only 1 species, i.e. with double zeroes (see above), sorted the remaining tables according to the particular species pair and spatial-temporal sampling unit involved, then tested each for departures from species sampling independence. Table II presents the results; 91% of tables contained sample sizes greater than 100. Statistically significant departures from independence appeared in 112 tables overall (54%). We considered the 4 *P. falciparum*–*P. ovale* tables too few to include in further analyses. In all but 1 of the 107 significant *P. falciparum*–*P. vivax* tables, the observed number of mixed-species infections was less than that expected. The converse held in all 5 significant *P. falciparum*–*P. malariae* tables. Among the 192 *P. falciparum*–*P. vivax* tables, the ratio of significant to nonsignificant tables shifted with the level of analysis, from 1:1 at level A, to 4:3 at B, 3:2 at C, and 2:1 at D. This did not appear to be true of the 13 tables pairing *P. falciparum* and *P. malariae*. We judged these sufficient for further analysis only at level A, however.

The deficit in mixed-species infections appears to be associated with overall prevalence of infection. Figure 1 and Figure 2 show distributional relationships between prevalence and *G*-test results for contingency tables within each sampling-unit group. The corresponding Mann–Whitney test results are: for the *P. falciparum*–*P. vivax* pair, for levels A and B, $P < 0.0001$, for level C, $0.0001 < P < 0.0005$, for level D, $0.001 < P < 0.005$; for *P. falciparum*–*P. malariae*, for level A (Fig. 2), $P > 0.1$. *G*-tests of the hypothesis that for *P. falciparum*–*P. vivax* an overall prevalence of 35% distinguishes nonsignificant from significant tables yield $P < 0.0001$ for all levels except D, for which $0.01 < P < 0.025$; for D, $0.005 < P < 0.01$ for a division at 30% prevalence. Transferring tables across rows or columns to attain $P > 0.01$ in these *G*-tests requires transfers equal to at least 28% of a marginal total for level A, 25% for B, 42% for C, and 9% for D. In sum, for the *P. falciparum*–*P. vivax* pair, this collection of tables shows a strong association between higher overall prevalences of infection and observed frequencies of mixed-species infection that are less than the product of individual species prevalences. This relationship does not appear to hold for *P. falciparum*–*P. malariae*, whereas for *P. falciparum*–*P. vivax*, it holds across 4 spatial-temporal levels of analysis.

A long-standing conservative rule of thumb with respect to contingency tables is that each cell count should be 5 or greater; values less than 5 may lead to too-frequent rejection of the null hypothesis (Sokal and Rohlf, 1981). Therefore, we used *G* and Mann–Whitney tests to check our results against the possibility of biased relationships between *G*-statistic significance and either the table sample sizes or the distributions of table entries with values less than 5 (and particularly zeroes). For $P < 0.01$ we found only 2 suggestions of such biases; in testing the categories 0–4 versus >4 for level A, we found significant deficits of entries in the significant 0–4 and nonsignificant >4 cells; in testing the categories 0 versus >0 for level B, we found significant deficits of entries in the significant 0 and nonsignificant >0 cells. In both cases, these biases would work against the principal patterns presented here.

Fox and Strickland (1989) noted that their conclusions relied on a prior demonstration by Fox et al. (1987) that the overall and species-specific prevalences found in surveys of village clinic attendees sufficiently mimicked those in community surveys. Because 16 of the *P. falciparum*–*P. vivax* studies used here acquired data either through clinics or with other techniques that collect blood only in cases of self-reported recent fevers, neither of which are random surveys of the population, we were concerned that the higher overall prevalence in these studies might bias our findings. To test for this possibility, we divided the contingency tables within each sampling unit into 2 subgroups, e.g., a clinical subgroup and a survey subgroup. The mean overall prevalence in the clinical subgroup was higher than the survey subgroup by 26% for level A, 14% for B, 29% for C, and 74% for D. We used the Mann–Whitney test to examine distributional differences between the constituent significant and nonsignificant categories within each subgroup, between the 2 significant categories across the subgroups, and between the 2 nonsignificant categories across the subgroups. All of the across-subgroup tests produced *P*-values greater than 0.025, and all but 1 of the within-subgroup tests produced *P*-values less than 0.01, with the sole discrepancy *P* = 0.025 for the comparison of significant and nonsignificant categories within the clinical subgroup for level D. That is, the association between higher overall prevalence and deficit of mixed-species infection holds for both subgroups.

Cohen's (1973) analysis considered overall prevalence as tied solely to geography and considered seasonality only as 1 of 4 elements of site "ecology." Richie (1988) considered variation in prevalence patterns over time at a given site. Few of the reports cited here allow further resolution of spatial, temporal, and age-related factors influencing overall and mixed-species infection prevalence; among those few we found no clear pattern, although data within each such report do support the prevalence-deficit relationship suggested above.

DISCUSSION

The key result of our analysis is that in human populations in which only *P. falciparum* and *P. vivax* are reported to cause malaria, high overall prevalence of infection is associated with significant deficits of dual infections. This association appears to be stronger and more general than suggested by our predecessors, whose analyses were based largely on studies in which 3 *Plasmodium* species were reported present. The lack of similar results for the *P. falciparum*–*P. malariae* pair and the lack of sensitivity to sample size argue that our result is not simply a byproduct of increases in microscopist workloads. Further-more, this association is apparently not completely explained by any of the additional factors we considered here, i.e., clinical status, site, month, year, or spatial-temporal scale of analysis.

Knowles and White (1930) recorded 15 instances that would meet our 2-species criteria, but only the report with the highest overall prevalence (73%) produces a contingency table significantly different from that expected; it shows a deficit of dual *P. falciparum*–*P. vivax* infections. Richie (1988) cites the unusual *P. malariae*–*P. vivax* report noted above, which yields tables with high overall prevalence (64–86%), but no significant departures from expected values. The only other 2-species report he cites shows 70% overall prevalence and a significant deficit of dual *P. falciparum*–*P. vivax* infections. The sole 2-species report used in Cohen's analysis (1973) involves *P. falciparum* and *P. malariae*; it shows a 3% overall prevalence and a significant deficit of dual infections. His section on "parasite rate," however, includes 3-species results that suggest that some overall prevalence between 30% and 47% might distinguish tables that are nonsignificant from those that are significant by his statistic "s" (see his table 1 and table 2). The remainder of his results address ecological factors, age groups, and spleen sizes; with a few exceptions (see especially table 5, from Peters and Standfast [1957]), they support the notion of a dividing line between 30 and 35% overall prevalence. Our results appear to be consistent with this reinterpretation.

Because prevalence describes frequencies of associations, it connotes a pattern of more specific biological interactions, for instance, at the levels of immunity, pathogenicity, or transmission. We do not know what processes generate the pattern reported here, but these seem to us the most critical areas for prospective empirical studies.

For instance, several older studies (Thomson, 1934; Wilson, 1936) mention the simultaneous presence of gametocytes of 2 (or more) species in dual infections; Shute (1946) remarked that gametocytes of both species were typically numerous in dual *P. falciparum*–*P. vivax* malariatherapy cases. Graves et al. (1988) noted that the presence of *P. falciparum* gametocytes appeared to reduce the infectivity of *P. vivax*, but not *P. malariae*, gametocytes when either was present concurrently. The studies we cite provide virtually no information regarding the transmission of mixed-species infections.

Thayer and Hewetson (1895) compared frequencies of inpatient admission and outpatient treatment to gauge the relative severities of *P. falciparum* and *P. vivax* infections; by this measure, mixed infections with the 2 putative species were more severe than infections with either alone. Unfortunately, few subsequent reports have examined the clinical implications of mixed-species malaria infections. One recent hospital study seems to support the notion that dual *P. falciparum*–*P. vivax* infection increases severity (Gopinathan and Subramanian, 1982, 1986) and another that there is no difference in clinical symptoms (Kremsner et al., 1989). Some of the data we extracted from a recent study of diagnostic criteria (Genton et al., 1994) suggest the possibility that mixed-species infections had a slightly beneficial effect, perhaps mediated by a higher prevalence of *P. malariae*; if so, this might fit the hypothesis of Black et al. (1994) that the incidence of fever in malaria and the presence of dual *P. falciparum*–*P. malariae* infection are inversely related.

Knowles and White (1930), Cohen (1988), and Richie (1973) each warned that the detection of prevalence involves densities and durations of infection as well as actual frequencies. Rosenberg et al. (1990) noted that in the Thai study, as at Garki, *P. falciparum* density was higher in dual than in single infections. In dual infections in the Pakistani study, the 2 species most often occurred at high or low densities simultaneously, which for Fox and Strickland (1989) argued against hypotheses of interspecific suppression (Mayne and Young, 1938; Molineaux et al., 1980). But again, these 2 reports are unique in the recent literature and, to the best of our knowledge, the most detailed information available about mixed-species infection dynamics in individual hosts remains malariatherapy charts. Figure 3 implies that even were all malaria infections mixed-species, a sampling of the intertwined patterns of dynamic infra- and suprapopulations could produce virtually any pattern of point-prevalence data. We suggest that in this sense some of the apparent deficits in mixed-species infections may represent both an artifact of method and a fact of parasite biology, rather than simply one or the other.

We expect that molecular-level detection methods will encourage empirical studies of these multilayered patterns and the biological processes that beget them, and that from these we will learn much more about the association of high overall prevalences with deficits of dual infections. Despite our relatively cautious, coarse-grained analytical approach, like our predecessors we remain wary of the many methodological variables that may confound results within a single field study as well as comparisons between studies. Accordingly, we hope that our work will not be mistaken as definitive, but will instead stimulate a program of prospective studies, sufficiently well planned and well executed to incorporate such factors as parasitemias and gametocytemias. Gametocytes embody a critical immunity-mediated link between parasite dynamics at the individual-host and host-population levels and are clearly among the plausible but underappreciated currencies of *Plasmodium* species interactions. Given that the volume of blood processed in detecting a human infection has typically been less than that in a mosquito

bloodmeal, the role of *Plasmodium* recruitment processes in mixed-species phenomena seems particularly opaque and intriguing.

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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 *Plasmodium falciparum*-*Plasmodium vivax*. Parts a-d show the results for levels of analysis A, B, C, and D, respectively (see text). 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) at that level.

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 *Plasmodium falciparum*-*Plasmodium malariae* for level of analysis A (see text). Conventions are as in Figure 1.

FIGURE 3.

An infection-history curve from the malariotherapy treatment of a neurosyphilis patient, case 220–1085 (Boyd and Kitchen, 1937). The vertical axis measures log 10 parasitized-erythrocyte (PE) density (per cc, from microscopy-based estimates); the horizontal axis counts days. The time course of *Plasmodium falciparum* asexual parasitemia (solid line) and gametocytemia (dashed line) and *Plasmodium vivax* asexual parasitemia (dotted line) is shown following inoculation of the patient (on day 0) by simultaneous feeding of 1 cage of *Anopheles stephensi* infectious for *P. falciparum* and another infectious for *P. vivax* (each infected for an unspecified period). The estimated detection threshold was 10 PE per cc (hence log 10 PE = 1).

TABLE I
A summary of recent reports of species occurrence in human populations and individuals.*

<i>Plasmodium</i> spp.	F	M	O	V	FM	FO	FV	MO	MV	OV	FMO	FMV	FOV	MOV	FMOV
Human population	X			X	X	X	X				X	X			X
Human individual	X	X	X	X	X	X	X	X	X		X	X			

* X indicates the existence of at least one published report. F, M, O, V indicate the occurrence (sole or combined) of *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*.

TABLE II

A summary of 209 contingency tables from 35 reports.*

Species	Reports with	Significant tables at level				Nonsignificant tables at level			
		A	B	C	D	A	B	C	D
F, M	A single table	4	0	0	0	3	1	0	0
F, O		1	0	0	0	1	0	0	0
F, V	Multiple tables	14	1	0	1	5	0	2	4
F, M		3	3	1	0	3	1	0	0
F, O		1	0	0	0	0	0	2	1
F, V		12	47	13	13	28	36	7	3
F, M	Total	7	1	1	0	6	2	0	0
F, O		2	0	0	0	1	0	2	1
F, V		26	32	13	14	33	36	9	7

* Abbreviations as in Table I; levels of analysis and significance are explained in the text. References:(1) Adak et al., 1994; (2) Ansari et al., 1986; (3) Arai et al., 1994; (4) Barker et al., 1989; (5) Choudhury et al., 1987; (6) de Arruda et al., 1986; (7) Dossou-Yovo et al., 1994; (8) Dutta and Bhattacharyya, 1990; (9, 10) Dutta et al., 1991,1994; (11) Ejezie et al., 1991; (12) Gazin et al., 1987; (13) Hedman et al., 1986; (14) Laserson et al., 1994; (15, 16) Malhotra et al., 1985a,1985b; (17) Mathur et al., 1992; (18) Nalin et al., 1985; (19, 20) Nosten et al., 1987,1991; (21) Prasad and Sharma, 1990; (22) Raccurt et al., 1993; (23) Ray et al., 1994; (24) Roche et al., 1991; (25) Rosenberg et al., 1990; (26) Schapira and da Costa, 1988; (27) Sharma et al., 1985; (28–30) Singh et al., 1988,1989, 1995; (31) Soro et al., 1989; (32, 33) Strickland et al., 1987,1988; (34) Warsame et al., 1989; (35) Yadav et al., 1993.

TABLE III

Data from 209 contingency tables.*

SPP	LVL	SAMP	PRV	SIG	REF	SPP	LVL	SAMP	PRV	SIG	REF
F, M	A	195	17	N	34	F, V	B	170	24	N	29
		798	18		22			893	24		29
		151	52		12			2,011	25		32
		150	75		13			257	27		29
		146	81		13			1,631	29		27
		124	82		7			784	31		27
		600	43	S*	31			1,685	33		27
		338	46		7			251	35		29
		666	58		31			648	36		27
		489	53	N	11			96	36		28
F, O	B	296	78		13	S	S	295	43		29
		1,266	51	S*	31			629	45		29
		462	55	S*	7			226	63		28
		189	58	N	26			1,694	15		29
		1,130	27	N	24			3,684	19		29
		185	61		24			2,957	20		29
		1,315	31	N	24			1,948	20		29
		251	5	N	32			3,980	20		29
		418	10		18			2,871	21		29
		1,178	11		32			3,563	22		29
F, V	C	1,076	12		32	S	S	791	23		27
		98	13		3			6,586	23		29
		442	14		32			2,323	24		29
		377	16		19			1,020	25		29
		587	17		32			2,677	25		29
		407	18		32			3,196	33		27
		58	19		16			3,877	34		27
		194	20		32			1,139	35		27
		912	22		32			1,997	35		27
		22	23		16			1,014	36		27
F, V	D	864	24		32	S	S	826	36		27
		39	26		16			466	36		29
		15	27		21			521	38		29
		52	27		16			395	38		29
		504	31		16			510	40		27
		885	32		15			458	43		28
		266	34		16			474	43		29
		333	37		32			553	44		28
		87	38		16			723	45		29
		321	41		15			156	47		28
F, V	A	207	43		16	S	S	281	47		28
		637	47		16			2,002	48		27
		61	51		16			691	48		27
		120	51		10			389	48		29
		1,698	53		16			452	48		29
		446	56		16			653	49		2
		244	60		16			864	50		29
		23	61		16			603	50		29
		70	61		2			251	52		27
		112	71		15			202	54		28
F, V	A	930	16	S*	32	S	S	1,423	55		27
		131	37	S	2			605	56		27
		179	40		32			298	59		29

SPP	LVL	SAMP	PRV	SIG	REF	SPP	LVL	SAMP	PRV	SIG	REF
		1,263	41		16			754	61		17
		200	43		2			928	61		29
		1,819	44		16			696	64		29
		155	45		2			637	68		29
		123	46		16			362	70		28
		914	47		16			274	74		28
		263	47		16			944	74		28
		1,974	49		16			311	76		2
		331	51		15			3,166	15	N	32
		168	51		16		C	831	17		30
		456	51		16			382	18		6
		1,025	53		16			2,381	19		32
		2,277	56		16			339	20		6
		1,226	56		16			1,182	22		32
		1,360	57		16			1,342	29		23
		3,313	59		16			322	31		6
		188	60		1			258	33		6
		83	60		15			826	25	S	4
		401	65		16			524	36		32
		588	65		16			571	40		4
		738	70		15			157	43		6
		124	70		15			1,860	44		25
		705	71		15			96	48		6
		167	71		2			225	50		2
		93	83		2			21,715	52		16
		61	84		2			261	52		2
		149	85		15			2,017	53		25
		397	86		15			224	56		2
		209	90		15			4,471	59		15
		1,065	1		29			254	73		2
		746	1		29		D	13,245	5	N	5
		755	2		29			612	13		9
		1,896	4		29			529	16		9
		101	5		29			7,353	18		32
		54	7		29			2,891	27		33
		184	8		29			190	27		8
		1,110	8		29			48	48		14
		1,545	9		29			55,121	17	S	29
		3,714	10		29			1,554	28		6
		1,900	10		29			1,435	29		9
		1,272	10		29			1,397	31		4
		2,646	10		29			25,515	35		27
		2,168	12		29			1,358	37		20
		2,699	13		32			7,309	46		35
		2,108	14		29			9,897	46		29
		753	14		27			15,473	53		35
		796	17		27			8,200	55		35
		2,643	17		32			12,209	57		35
		714	18		27			964	58		2
		150	20		29			2,510	59		28
		182	23		27			1,042	59		28
		309	23		29						

* Abbreviations: species, SPP; level of analysis, LVL; sample size, SAMP; overall prevalence, PRV (%); and statistical significance, SIG; where S indicates a table with an observed prevalence of mixed-species infection < the product of individual-species prevalences, S* the converse, and N a table with mixed-species prevalence equal to that product. Other abbreviations and reference (REF) numbers as in Table II.