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Systematic review and meta-analysis of the interaction between *Plasmodium falciparum* and *Plasmodium vivax* in humans

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Abstract

Objectives: This study aimed to quantify the interaction between *Plasmodium falciparum* and *P. vivax* and the sources of heterogeneity between studies.

Methods: We systematically reviewed three databases: Medline (1966–2001), Embase (1980–2001) and CAB-health (1976–2001). Random effects meta-analysis was applied to the data of 62 selected populations. Meta-regression was used to assess the following potential sources of heterogeneity: age-group, presence of fever, continent, temporal and spatial span of studies, and endemicity level.

Results: The summary odds ratio (OR) between *P. falciparum* and *P. vivax* was 0.6 (95% CI: 0.49–0.79). The minimum and maximum observed ORs were 0.01 and 10.9, respectively, and the heterogeneity test was highly significant ($\tau^2 = 0.92$, $p<0.0001$)—the ORs varied over a very wide range. The ORs in longer studies and in those from areas with higher prevalence yielded smaller, more strongly negative association. This is consistent with the idea that any difference in the species' temporal patterns should decrease the OR, and more so over longer periods of time.

Interpretation & conclusion: Although such odds ratios between *Plasmodium* species may be partly due to missed mixed infections when reading blood slides, the negative association between the OR and prevalence supports the existence of biological interactions such as suppression or cross-immunity between species.

Key words Meta-analysis – *Plasmodium* – species interactions – systematic review

Introduction

Dual infections with *Plasmodium* spp are reported in many studies. Results of these studies generally do not support the hypothesis of species sampling independence such as no interaction between species, and a wide range of associations has been reported[1]. Howard *et al*[2] used a log linear regression model to show the association between multiple species parasite infections. They showed that the natural logarithm of the odds ratio between *Plasmodium falciparum* and *P. vivax* varied over a wide range from –5.08 (in Bangladesh) to +2.56 (in Sierra Leone). In addition, they found that for Asian countries the associations were largely negative; however, positive associations were seen in Tanzania, Papua New Guinea and the United States of America (USA)[2]. Some part of these variations could be explained by the differences in human susceptibility to the *Plasmodium* species among populations.

Human *Plasmodium* spp share the same transmission

route. In addition, some subgroups within a population, such as children and pregnant women, are more susceptible to all species because of lower levels of immunity. Therefore, based upon the above two facts, a positive correlation between these infections might be expected[3–5]. Nonetheless, according to the results of most prevalence surveys, fewer mixed-species infections were observed than would be expected based on the product of the frequencies of individual species. This finding suggests that one parasite has excluded another or suppressed its parasitaemia to undetectable levels[6–9]. However, underestimation of the number of mixed infections based on microscopy is an alternative explanation[8,10].

Nevertheless, there has not been a systematic analysis of the published data to see how far they bear out the alternative explanations for species associations. We, therefore, performed a systematic review of the literature, and a meta-analysis to estimate the interaction between *P. falciparum* and *P. vivax*, and assess possible sources of heterogeneity, including age, the geographical and temporal span of each study, and the prevalence of malaria.

Methods

Literature search and study selection: In the first stage, Medline (1966–2001), Embase (1980–2001) and CAB-health (1976–2001) were searched systematically with wide key words to optimise the sensitivity of data collection. This search selected 829 citations. In the next stage, using a proforma checklist, information about study objectives, sampling methods, type of study and main findings were extracted from the abstracts. Based on this information some ineligible papers were excluded. The eligible studies were those which reported the frequencies of *P. falciparum, P. vivax* and mixed infections among random or pseudo-random samples in a defined population. However, studies which included immigrants from endemic areas, or assessed a special subpopulation such as military personnel, were excluded. Out of 829 abstracts, 104 (12.5%) papers were categorised as potentially eligible for meta-analysis, and the eligibility of 68 (8.2%) more was checked by reviewing their full texts. Those papers which chose non-random samples, or estimated neither the incidence nor the prevalence of malaria were excluded. In addition, reports from national-level surveillance systems were excluded because it was anticipated that their validity would be lower than that of research studies, particularly in terms of detecting mixed infections.

In the last stage, the full texts of 172 papers were reviewed and the data of considered to be eligible papers were abstracted. Out of 104 papers whose abstracts had been classified as potentially eligible, 42 were eligible (40.4%). Out of 68 papers whose abstract was insufficient to decide potential eligibility, 18 (26.4%) papers were eligible. One paper in Chinese was excluded, but papers in other languages (French, Portuguese and Arabic) were assessed. Three papers reported the frequency of infections in more than one population. These papers recruited distinct populations in different geographical locations. The data for these populations were treated independently.

The abstracted data from the full texts of eligible papers included: the duration of the studies; their geographical spans and locations; the age group of subjects; subjects' disease status (normal or febrile); the number of examined blood slides; and the number of slides positive for *P. falciparum* only, for *P. vivax* only, and for both species.

Statistical analysis: Using random effects meta-analysis, a summary odds ratio (OR) was estimated. It was then determined how much of the observed variation between studies could be explained by age group, continent, endemicity, geographical size of study areas, and duration of studies. Eight studies reported absence of mixed infections. To allow their inclusion in the logarithmic scale analysis, Haldane's estimator was obtained by adding 0.5 to all cells[11].

Using Stata 8, weighted regression was used to assess the effect of explanatory variables on the heterogeneity between studies. An additive term was utilised to estimate the residual heterogeneity (τ^2: tau-square) which is between-study variance of the $\log_e$-odds ratio. This term was estimated by the moment method[12].

In order to assess possible publication bias, the Begg and Mazumdar test was used: this is based on the adjusted rank correlation between the effect estimates and their variances[13]. In meta-analysis, some small studies may show extreme results. Bayesian techniques can be used to adjust the point estimations of individual studies. This involves computing an a posteriori estimate of the log-OR for each study, equal to the estimate from the individual study with the weighting average of the meta-analysis summary estimate and the individual study's estimate, with the weighting being based on their variances. This technique was used to adjust the range of observed ORs. In this approach, a normal distribution with mean equal to the overall log-OR, and variance equal to τ^2, was used as the Bayesian prior distribution for the log-OR of each study[14].

Results

Data from 62 populations were analysed. More than 80% were in Asia (n = 52); 8.1% selected only children (n = 5) and 58.1% normal (non-febrile) subjects (n = 36). The minimum and maximum numbers of examined slides per population were 95 and 986,127, respectively (mean 23,058). Eight studies did not find any mixed infections, while one study found 782 (mean 33).

The minimum and maximum observed ORs were 0.02 and 10.9, respectively (Table 1)[15–69]. Using the Bayesian adjustment, to allow for sampling variation in small studies, narrowed this range considerably (0.18–5.7).

Out of 62 studies, 41 (66.1%) showed an OR less than one (i.e. negative species association); but only 20 (48.8%) of them were statistically significant. Among the 21 studies with OR greater than one, 8 (38.1%) had significant p-value. It implies that the results of these studies were not consistent, some of them showed positive and some of them showed negative interaction between species.

There were significant associations between the ORs and prevalences of *Plasmodium* spp. Pearson correlation coefficients between the ORs and the prevalences of *P. falciparum, P. vivax* and all species were –0.33, –0.34 and –0.44, respectively ($p<0.0001$). These results showed that the ORs were negatively associated with the species prevalence—the species interaction was more negative in higher prevalence areas.

Lower summary ORs were observed in studies with higher prevalences of infections. The OR in studies with prevalence of infection 30% or more, considering both species together, was 0.32 (95% CI: 0.22–0.47); while the corresponding OR in studies with prevalence less than 15% was 2.51 (95% CI: 1.66–3.8). Similar descending trends in the ORs were observed classified by the prevalences of *P. falciparum* and *P. vivax* infections, when the species were considered separately. Therefore, some part of the inconsistency between findings of studies could be explained by the variation of *Plasmodium* spp.

The overall OR was 0.6 (95% CI: 0.46–0.8) (Fig. 1). The overall τ^2 (the variance of $\log_e$-OR between populations) was 0.91. It was even greater than this in many subsets of studies classified by explanatory variables (Table 2). This finding again supports that a wide range of interaction between species was existing among studies.

The summary OR in the South American studies was significantly lower than those in other continents: 0.21, 0.62 and 1.76 in South America, Asia and Africa respectively. Although the difference between the ORs in Africa and Asia was considerable, it was not statistically significant which was mainly due to

Table 1. The odds ratio between *P. falciparum* and *P. vivax* positive; studies are sorted by their year of publication

Author(s); year	Odds ratio (95% CI)	Author(s); year	Odds ratio (95% CI)
Cross *et al* 1975[15]	1.53 (0.37–6.41)	Giboda *et al* 1992[41]	0.16 (0.08–0.33)
Maffi *et al* 1975[16]	4.43 (1.31–15.04)	Anthony *et al* 1992[42]	0.03 (0–0.44)
Rajagopal 1976[17]	1.94 (0.12–32.75)	Syafruddin *et al* 1992[43]	0.28 (0.16–0.5)
Cross *et al* 1976[18]	0.43 (0.16–1.17)	Adak *et al* 1994[44]	1.2 (0.26–5.6)
Smrkovski *et al* 1982[19]	10.92 (3.39–35.2)	Dutta *et al* 1994[45]	0.5 (0.12–2.02)
Cattani *et al* 1983[20]	0.44 (0.05–3.57)	Rafi *et al* 1994[46]	0.86 (0.24–3.1)
Hii *et al* 1985[21]	1.14 (0.67–1.94)	Das *et al* 1994[47]	0.71 (0.1–5.09)
Hii *et al* 1985[21]	5.16 (1–26.72)	Mizushima *et al* 1994[48]	0.1 (0.03–0.34)
DeArruda *et al* 1986[22]	0.22 (0.03–1.61)	Gautret *et al* 1995[49]	0.84 (0.56–1.28)
Cattani *et al* 1986[23]	2.91 (0.16–51.4)	Ghosh & Yadav 1995[50]	1.75 (0.1–31.35)
Verma & Srivastava 1986[24]	0.46 (0.4–0.53)	Dietze *et al* 1995[51]	0.39 (0.22–0.72)
Beljaev *et al* 1987[25]	0.15 (0.07–0.35)	Dutta & Mahanta 1995[52]	0.54 (0.12–2.33)
Strickland *et al* 1987[26]	0.3 (0.17–0.51)	Uchida *et al* 1995[53]	0.16 (0.01–2.75)
Strickland *et al* 1987[26]	1.17 (0.74–1.85)	Sherchand *et al* 1995[54]	0.3 (0.13–0.69)
Strickland *et al* 1987[26]	0.6 (0.31–1.14)	Das *et al* 1997[55]	0.19 (0.17–0.21)
Strickland *et al* 1987[26]	1.73 (1.04–2.86)	Hansmann *et al* 1997[56]	0.92 (0.85–0.99)
Singh & Sharma 1989[27]	0.79 (0.57–1.08)	Belizario *et al* 1997[57]	0.07 (0.01–0.56)
Singh & Sharma 1989[27]	0.94 (0.75–1.17)	Belizario *et al* 1997[57]	9.56 (4.5–20.32)
Dutta *et al* 1989[28]	0.13 (0.03–0.58)	Belizario *et al* 1997[57]	2.58 (0.35–18.94)
Itokawa *et al* 1989[29]	0.97 (0.69–1.37)	Seboxa & Snow 1997[58]	1.68 (0.71–3.98)
Ghosh *et al* 1989[30]	0.05 (0.03–0.07)	Carney *et al* 1977[59]	0.95 (0.13–7.04)
Graves *et al* 1989[31]	0.1 (0.04–0.3)	Mandal *et al* 1998[60]	0.02 (0.01–0.04)
Fox & Strickland 1989[32]	0.16 (0.01–2.73)	Joshi *et al* 1998[61]	0.14 (0.03–0.59)
Das *et al* 1989[33]	0.83 (0.33–2.07)	Camargo *et al* 1999[62]	0.34 (0.08–1.55)
Dutta & Bhattacharyya 1990[34]	0.24 (0.01–4.4)	Singh *et al* 2000[63]	2.0 (0.93–4.31)
Rajagopalan *et al* 1990[35]	0.32 (0.04–2.59)	Hozhabri *et al* 2000[64]	3.49 (1.1–11.09)
Nosten *et al* 1991[36]	2.75 (1.62–4.64)	Mehlotra *et al* 2000[65]	0.17 (0.11–0.27)
Subramanian *et al* 1991[37]	3.11 (1.11–8.72)	Srivastava & Yadav 2000[66]	1.36 (0.98–1.88)
Moitinho *et al* 1991[38]	0.34 (0.19–0.61)	Pinto *et al* 2000[67]	1.24 (0.07–22.18)
Gordon *et al* 1991[39]	0.31 (0.15–0.62)	Roper *et al* 2000[68]	0.65 (0.24–1.76)
Dutta *et al* 1991[40]	3.09 (1.42–6.73)	Singh *et al* 2001[69]	0.51 (0.18–1.39)

the small number of studies from Africa; hence, we should interpret this finding with cautiousness.

The lower summary OR in South American studies may be explained by the higher *P. vivax* prevalences. The average of *P. vivax* prevalence in South American studies was 20.2% (SD = 10.6); the corresponding values in African and Asian studies were 6.7 (SD = 7.3) and 6.5 (SD = 4.7) respectively. Therefore, lower ORs in South America may be explained by negative association between the prevalence of *P. vivax* infection and the OR.

The summary OR in studies that recruited only children was greater than that in studies which recruited all age groups (1.28 vs 0.56). The confidence interval around the OR in children was very wide (0.31–6.08) which may be associated with the small number of available studies (n = 5) and considerable residual heterogeneity ($\tau^2 = 2.14$). The summary OR of studies in normal subjects was around twice of that in febrile subjects (0.9 vs 0.35; p = 0.04). In other words, the species interaction was more strongly negative in febrile cases than in normal subjects.

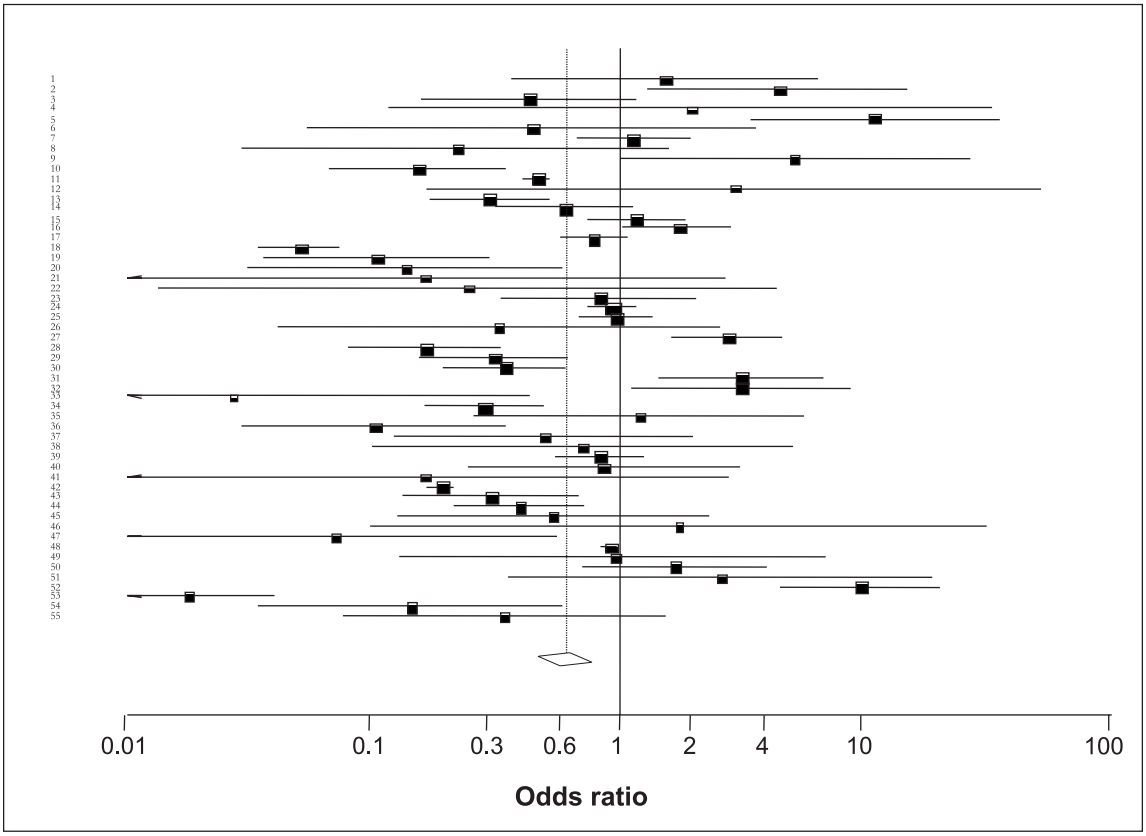

Fig. 1: Forest plot of the OR between *P. vivax* and *P. falciparum* using a random effect model to estimate the 95% confidence interval around the summary OR (the diamond). Each square represents the point estimate of one study, with the area being proportional to the study weight. Each horizontal line represents the 95% confidence interval for a single study. Arrows indicate confidence intervals which extend beyond the scale

The difference between summary ORs in febrile and normal subjects can also be explained by differences in the prevalence of *Plasmodium* spp. The average prevalences of infections in febrile and normal subjects were 30.2% (SD = 19.7) and 21% (SD = 13.5), respectively. This was probably due to the difference in the prevalence of *P. falciparum* infection (22.7% vs 15.1%). Furthermore, the prevalence of infection in studies among children was lower than those in mixed age group studies (7.9%, SD = 5.7 vs 19.2%, SD = 15.6). Therefore, the greater summary OR in the former group may, again, be due to the lower prevalence of infections.

The meta-regression results showed that only *P. falciparum* and *P. vivax* prevalence ($p<0.001$) and temporal span of studies ($p = 0.03$) had significant association with the OR, and explained some part of the residual heterogeneity ($\tau^2 = 0.72$, vs $\tau^2 = 0.91$ in the model without any explanatory variable). The direction of the regression associations was consistent with the patterns already noted from Table 2. The association between species was more strongly negative in studies with higher prevalence of infection, and with a greater temporal range.

The results of a Begg and Mazumdar[13] adjusted rank correlation test did not support the possibility of publication bias ($p = 0.96$).

Discussion

This systematic literature review included only pa-

Table 2. The odds ratio of *P. vivax* as risk factor of *P. falciparum* classified by continent, age group, study subjects, temporal and spatial span and the frequencies of species among examined slides

Subgroup (No. of studies)	Odds ratio (95% CI)	p-value of heterogeneity	τ^2
Continent			
Asia (52)	0.62 (0.46–0.83)	<0.0001	0.8
South America (6)	0.21 (0.16–0.26)	0.0013	0.59
Africa (4)	1.76 (0.47–6.6)	<0.0001	1.68
Age group			
Children (5)	1.38 (0.31–6.08)	<0.0001	2.14
Mixed (57)	0.56 (0.43–0.75)	<0.0001	0.82
Subjects			
Normal (36)	0.9 (0.65–1.24)	<0.0001	0.68
Febrile (26)	0.35 (0.21–0.58)	<0.0001	1.33
Spatial span			
A few villages (36)	0.5 (0.33–0.75)	<0.0001	1.14
District (16)	0.99 (0.591–1.63)	<0.0001	0.73
Larger than a district (10)	0.49 (0.3–0.82)	<0.0001	0.43
Temporal span			
Month (26)	0.81 (0.56–1.17)	<0.0001	0.55
Season (12)	0.97 (0.52–1.79)	<0.0001	0.66
Year or longer (24)	0.39 (0.26–0.6)	<0.0001	0.88
P. falciparum risk (%)			
<10 (23)	1.06 (0.54–2.1)	<0.0001	5.15
10–14.99 (10)	0.75 (0.42–1.35)	<0.0001	0.6
≥15(29)	0.4 (0.28–0.57)	<0.0001	0.77
P. vivax risk (%)			
<5 (27)	1.43 (0.98–2.1)	<0.0001	0.5
5–9.99 (18)	0.49 (0.32–0.75)	<0.0001	0.6
≥10 (17)	0.25 (0.13–0.5)	<0.0001	1.72
Both species risk (%)			
<15 (18)	2.51 (1.66–3.8)	0.0021	0.36
15–29.99 (22)	0.5 (0.36–0.7)	<0.0001	0.37
≥30 (22)	0.32 (0.22–0.47)	<0.0001	0.62
All studies (62)	0.6 (0.46–0.8)	<0.0001	0.91

pers published after 1966. Estimation of the frequency of mixed infections was hardly ever their main objective, so it seems unlikely that the probability of publication was associated with this frequency. So publication bias should not be a major concern for our meta-analysis. This conclusion was supported by the non-significance of the statistical test for publication bias (Begg and Mazumdar[13] adjusted rank correlation test, p = 0.96). Our study did not include "grey literature" such as the reports of health ministries based on national surveillance data. However, since missed mixed infections are usually more common in routine surveillance data, it is expected that adding those surveillance data would push the ORs even toward further zero.

The number of papers which were included in this meta-analysis was high (n = 62) and allowed us to explore the

sources of heterogeneity among the ORs. The OR of *P. vivax* and *P. falciparum* varied over a wide range: from 0.02 to 10.9 (Bayesian range: 0.18, 5.7). The summary OR was 0.6 (random effect 95% CI: 0.46–0.79). It meant that, overall, infection with one species decreased the risk of infection with the other. The ORs in studies that recruited children, or subjects without fever, were greater. Moreover, the summary OR of studies from South America was significantly lower than those in studies from Asia or Africa. However, all these differences could be explained based on the differences in the frequencies of infections.

Consistent with this hypothesis, the regression modelling showed that a considerable part of the heterogeneity between ORs can be explained by the frequencies of *Plasmodium* spp among the blood slides examined, and the temporal span of studies. The residual heterogeneity between ORs of all studies was 0.91; having taken into account of these two factors, it dropped to 0.72.

A group at high risk for one species might not have high infection risk for the other species simultaneously. Therefore, discrepancies between temporal and spatial variations of *Plasmodium* spp risks, e.g. different seasonal patterns, were one of the possible explanations for negative interaction between species. Such discrepancies might be expected to be greater in larger studies which observed people over a longer period or wider area. This reasoning can explain the observed negative association between the temporal span of studies and the OR. Although Howard *et al*[2] did not systematically review the literature, and assessed the associations between several different parasitic infections, their results on *Plasmodium* spp may also be explicable in terms of prevalence of infection.

The crude ORs in febrile subjects were less than that in normal subjects (0.35 vs 0.9 respectively). Acquired immunity against malaria has negative correlation with fever[70–72]. Therefore, it may be implied that negative association between species was stronger in those who had acquired immunity. However, the above conclusion is not compatible with the differences between crude ORs in studies that recruited children and mixed age groups (1.38 vs 0.56 respectively). Children usually have less immunity while their summary OR was higher and this showed that there is a positive association between species in the young age group. This contradiction may again be explained by the frequency of infections. In the regression models, the frequencies of infections were the only significant variables which explained a considerable part of the residual heterogeneity. Having taken into account the frequencies, the effects of age-group and presence of fever were not significant. There were also clear differences between the frequencies of infections in subsets of studies which recruited in febrile and normal subjects, and studies which surveyed children and all age groups. The OR was lower in studies with high infection frequencies and the effects of age and fever could be explained based on the differences in frequencies of infections.

McKenzie and Bossert[73] showed that high overall prevalence of infection is associated with significant deficits of dual infections based on the product of individual species prevalences. They explained this finding based on biological interactions between species without any specific suggestion about mechanisms of the interactions. Some of these findings were similar to that of our meta-analysis although our analysis was based on the odds ratio not the product of prevalences i.e. risk ratio[73]. In addition, our analysis assesses the effects of temporal and spatial span of studies and other possible explanatory variables.

Regarding the possible explanations for species interactions, the differences between group- and individual-level data should be noted. Both the reviews by McKenzie and Bossert[73] and the current study used group-level data i.e. ecological fallacy. However, the association of the OR with prevalence sug-

gests that an important role is also played by within-host biological processes, such as one species suppressing another, or acquired immunity, if the latter confers more protection against mixed than single infections.

Although an overall negative interaction between *P. vivax* and *P. falciparum* was observed in published studies, there was a considerable heterogeneity. Among the available covariates, the temporal span of each study, and species frequencies, explained most of this heterogeneity. The observed negative association between the prevalence and ORs may imply that a biological interaction like suppression or cross-immunity between species reduced the summary OR. However, further studies are needed to identify with more certainity the mechanisms behind these associations.

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