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Prevalence and proportion of *Plasmodium* spp. triple mixed infections compared with double mixed infections: a systematic review and meta-analysis

Manas Kotepui^{1*}, Kwuntida Uthaisar Kotepui¹, Giovanni D. Milanez² and Frederick R. Masangkay²

Abstract

Background: Although mixed infection by two *Plasmodium* species has been recognized, mixed infection by three different *Plasmodium* species within one individual has not been clarified. This study sought to determine the pooled prevalence and proportion of triple mixed *Plasmodium* spp. infection compared with double mixed infection.

Methods: Articles from PubMed, Scopus, and Web of Science were searched for cross-sectional studies of triple mixed infection by *Plasmodium* species and then were retrieved and extracted. The pooled proportion and prevalence of triple mixed infection by *Plasmodium* species were subjected to random-effects analysis. The secondary outcomes were differences in the pooled proportion between triple mixed infection and double mixed infection by *Plasmodium* species.

Results: Of 5621 identified studies, triple mixed infection data were available for 35 records, including 601 patients from 22 countries. The overall pooled prevalence of triple mixed infection was 4% (95% Confidence Interval (CI) 3–5%; $l^2 = 92.5\%$). The pooled proportion of triple mixed infection compared with double mixed infection was 12% (95% CI 9–18; $l^2 = 91\%$). Most of the included studies (29/35; 82.9%) presented a lower proportion of triple mixed infection than double mixed infection. Subgroup analysis demonstrated that the proportion of triple mixed infection was the highest in Oceania (23%; 95% CI 15–36%) and Europe (21%; 95% CI 5–86%), but the lowest in the USA (3%; 95% CI 2–4%). Moreover, the proportion of triple mixed infection was higher in residents (20%; 95% CI 14–29%) than in febrile patients (7%; 95% CI 4–13%), when compared with the proportion of double mixed infection. Subgroup analysis of the age groups demonstrated that, compared with the proportion of double mixed infection, triple mixed infection was lower in patients aged ≤ 5 years (OR = 0.27; 95% CI 0.13–0.56; $l^2 = 31\%$) and > 5 years (OR = 0.09; 95% CI 0.04–0.25, $l^2 = 78\%$).

Conclusions: The present study suggested that, in areas where triple mixed infection were endemic, PCR or molecular diagnosis for all residents in communities where malaria is submicroscopic can provide prevalence data and intervention measures, as well as prevent disease transmission and enhance malaria elimination efforts.

Keywords: Plasmodium, Mixed infection, Triple infection, Quadruple infection, Concurrent infection

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Background

Malaria remains a major disease of public health concern worldwide, particularly in sub-Saharan Africa [1]. In 2018, the World Health Organization (WHO) estimated 228 million cases and 405,000 deaths caused by malaria

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worldwide, mostly in children aged younger than 5 years [1]. Infections of the *Plasmodium* species usually present as monoinfection by one species; however, mixed infections by more than one species within one individual can occur [2–6]. The interactions between mixed infections are not well characterized, but may play roles in disease progression and outcomes [7]. Moreover, mixed infections by *Plasmodium* species are often not recognized or are underestimated by microscopists [8]. In Asia, mixed infections by *Plasmodium* species have occurred at a frequency of 2% to 30% [9]. Although mixed infection by two *Plasmodium* species has been recognized, mixed infection by three different *Plasmodium* species within one individual has not been clarified.

This study sought to determine the pooled prevalence and proportion of triple mixed infection by *Plasmodium* spp. compared with double mixed infection. This information is necessary to guide the progress of research on mixed infection and malaria management, as well as control strategies for strategic malaria diagnostic service choices and treatment options.

Methods

Search strategy

Articles from PubMed, Scopus, and Web of Science were searched for cross-sectional studies on triple mixed infection by *Plasmodium* species in patients with all species of malaria. Triple mixed infection were defined as infection with three *Plasmodium* species. Articles published between February 2, 1907, and February 24, 2020, in the English language were included in the analysis if they explicitly reported the presence of triple mixed infection by *Plasmodium* species. The search strategy included the search terms "(Plasmodium OR Malaria) AND ("Mixed infection" OR "Triple infection")" (Additional file 1: Table S1).

Selection criteria

Observational studies, prospective cohorts, and casecontrol designs were included if they reported triple mixed malaria infection among the included participants by polymerase chain reaction (PCR) or molecular methods. Studies were excluded if the numbers of triple mixed infection could not be extracted and if only one species of *Plasmodium* was studied or evaluated subsequently from microscopy or rapid diagnostic test (RDT). Animal studies, clinical drug trials, case reports, experimental studies, reviews, systematic reviews, and polymorphism studies were excluded because they were considered incompatible study designs for the present review and meta-analysis. Studies were selected and identified by two independent authors (MK and KUK), with discrepancies resolved following discussion with a third author (FM). The protocol of this analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Data extraction and definitions

The data extracted for individual studies included author names, year of publication, study area, year of study, details, and numbers of the participants, age ranges, blood collection methods, DNA extraction method, investigated gene, PCR method, malaria positivity status, number of double mixed infections, and number of triple mixed infections. The numbers of patients with four different *Plasmodium* species were also extracted for further discussions in the present study. Subgroup analyses were performed for the following parameters: publication year (before and after 2000), continent (Asia, Africa, America, Europe, or Oceania), participant group (febrile patients and residents from the same community), type of blood storage for PCR (EDTA blood or dried blood spots), and age group of patients with mixed infection.

Data analysis

The primary outcome was the pooled proportion and pooled prevalence of triple mixed infection by *Plasmodium* species, with random-effects meta-regression used to investigate these pooled analyses. The analyses were performed using Stata Statistical Software (Release 15; StataCorp LLC. USA). The secondary outcomes were differences in the pooled proportion between triple mixed infection and dual mixed infection by *Plasmodium* species reported in the included studies. Those differences were estimated using random-effects meta-analysis to calculate the odds ratio (ORs) and 95% confidence intervals (CI). The analyses were performed using Review Manager 5 (RevMan 5, Cochrane Community).

Potential bias related to individual studies was assessed using a tool developed by the Newcastle-Ottawa Scale (NOS) to assessing the quality of non-randomized studies in meta-analyses. The quality of included studies was rated if they qualified with a maximum of 7 stars. Publication bias related to study effects was assessed by funnel plot asymmetry. Between-study heterogeneity was assessed by the I² statistic and was assessed using the random-effects statistic. Subgroup analysis of the baseline characteristics included continent (Asia, Africa, America, Europe, or Oceania), participants (residents and febrile individuals), type of blood storage for PCR (EDTA blood or dried blood spots), and age group. For the subgroup analyses of age groups and different mixed infections (double and triple infection), the age groups of patients were classified as ≤ 5 and > 5 years because children younger than 5 years are one of the most vulnerable groups affected by malaria [10].

Characteristics of the included studies

After screening the titles and abstracts of 5621 identified studies published between February 2, 1907, and February 24, 2020, the full texts of 344 (6.11%) potentially

relevant studies were reviewed (Fig. 1). There were 309 studies that did not meet the inclusion criteria, mostly because they did not document triple mixed infection by *Plasmodium* species in their studies. Thirty-five (10.1%) of 344 studies could be extracted and were included in



the analysis (Table 1). Of the 35 studies, 14 (40%) were from the Asia-Pacific region, 9 (25.7%) were from Africa, 5 (14.3%) were from Europe, 4 (11.4%) were from Oceania, and three (8.6%) were from the (Table 1). Among 35 included studies from 22 different countries, most (4/35, 11.4%) were from Papua New Guinea [11-14], Cambodia [15-17], India [6, 18, 19], Italy [4, 20, 21], and Uganda [2, 22, 23]. Most of the participants included among the studies were residents (16/35, 45.7%), febrile patients (11/35, 31.4%), and malaria-positive cases (6/35, 17.1%). Twenty-four studies (68.6%) reported age ranges, whereas others did not. One study used samples from doubtful microscopic examination [18], whereas another enrolled both febrile and asymptomatic patients to perform PCR analysis [6]. More than half of the included studies (18/35, 51.4%) used EDTA blood to extract the DNA for PCR analysis, whereas others used dried blood spots (15/35, 42.9%), and one study used thick smears for DNA extraction [24]. Most of the studies (28/35, 80%) used DNA commercial kits, while four studies (4/35, 11.4%) used 30% Chelex-100 and phenol-chloroform extraction for DNA extraction. All of the included studies used the 18S ribosomal RNA (rRNA) gene to identify the Plasmodium genus and species. Overall, 44,310 participants were enrolled in the included studies. Of those, most were residents (34,483, 77.8%), febrile patients (7797, 17.6%), and malaria-positive samples (1675, 3.78%).

Regarding the number of malaria-positive participants by PCR, 12,023 patients were infected by one of the five Plasmodium species (Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi). Among those positive patients, 3059 (25.4%) were infected with two different Plasmodium species. The most common types of mixed infection were P. falciparum and P. vivax (1318, 11%), P. falciparum and P. malariae (775, 6.4%), and P. vivax and P. malariae (645, 5.4%). Among those 12,023 positive patients, 601 (5%) were infected with three different Plasmodium species. The most common types of mixed infection were P. falciparum/P. malariae/P. vivax (355, 3%), P. falciparum/P. malariae/P. ovale (137, 1.1%), and P. falciparum/P. vivax/P. ovale (83, 0.7%). Fifty-three patients (0.44%) had quadruple mixed infection with P. falciparum/P. vivax/P. malariae/P. ovale.

Quadruple mixed infection was identified among the present studies and comprised of *P. falciparum/P. vivax/P. malariae/P. ovale* in one individual. Fifty-three patients (0.44%) had quadruple mixed infection. Most (40/53, 75.5%) were found in Papua New Guinea [11–14], followed by Cambodia (12/53, 22.6%) [15–17], Thailand (8/53, 15%) [24, 25], India (1/53, 1.9%) [18], China (1/53, 1.9%) [26], and Laos (1/53, 1.9%) [27].

Quality of the included studies

All of the included studies were rated with a maximum of 7 stars (Table 2). Sixteen studies received 7 stars, 12 received 6 stars, and 7 received 5 stars. The twelve studies rated with 6 stars used febrile controls, and the 7 studies rated with 5 stars used malaria-positive samples for PCR analysis.

The pooled prevalence of triple mixed infection

The numbers of triple mixed infection were available for 35 records that included 601 patients from 22 countries. The overall pooled prevalence of triple mixed infection (4%; 95% CI 3–5%; $I^2 = 92.5\%$) with no evidence of publication bias related to small study effects is shown in the funnel plot (Fig. 2). The highest prevalence of triple mixed infection for an individual study was 46% (95% CI 37–55) in a study by Zhou et al. [24].

Comparison of the pooled proportion of triple and double mixed infections

The pooled proportion of triple mixed infection compared with double mixed infection was 12% (95% CI 9–18, $I^2=91\%$) (Fig. 3). Most of the included studies (29/35, 82.9%) presented a lower proportion of triple mixed infection than double mixed infection. Only one study demonstrated a higher proportion of triple mixed infection than double mixed infection [24]. Another included study by Peruzzi et al. could not input the present meta-analysis because it had only reported on triple mixed infection, not double mixed infection [21].

Subgroup analysis

Subgroup analysis of the continents from 34 studies where triple mixed infection were reported in the included studies was available. The analysis demonstrated that the proportion of triple mixed infection was the highest in Oceania 23% (95% CI 15–36%) and Europe 21% (95% CI 5–86%) compared with that of double mixed infection (Fig. 4). However, the proportion of triple mixed infection was the lowest in America (3%; 95% CI 2–4%). A subgroup difference was found between continents with a high level of heterogeneity (P-value < 0.0001, $I^2 = 94.8\%$).

Subgroup analysis of the febrile subjects and residents from 27 studies was available (Fig. 5). Compared with the proportion of double mixed infection, triple mixed infection was higher in residents (20%; 95% CI 14–29%) than in febrile patients (7%; 95% CI 4–13%). A subgroup difference was observed between febrile patients and residents with a high level of heterogeneity (P-value = 0.004, I^2 =88.2%). Subgroup analysis of the blood collection method for PCR from 32 studies was available. The proportion of triple mixed infection using EDTA blood was

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No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups (n)	Blood storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
	Asua et al. [2]	Uganda	Malaria posi- tive (499)	6 months to 10 years		Dried blood spots	Chelex extrac- tion kits	185 rRNA	Nested PCR	474	 Pf/Pm (19) Pf/Po (14) Pf/Pv (3) Pm/Pv (1) Total = 37 	Pf/Pm/Po (1)
\sim	Barber et al. [3]	Malaysia (2009–2011)	Malaria posi- tive (653)	P. knowlesi (IQR 20–50 years), 9–31 years), P. vivax (19 years, IQR 7–32 years)	Triple infection: <5 tion: <5 (1), 10–14 (1), 30–34 (1), 80–84 (1), 80–84 (1), 80–84 (1), 80–84 (1), 80–34 (2), 10–14 (5), 15–19 (3), 20–24 (5), 15–19 (3), 20–24 (4), 35–39 (3), 20–24 (4), 35–39 (3), 40–44 (5), 75–79 (1), 75–79 (1), 75–79	₹ Z	₹ _Z	185 FRNA	Nested PCR	44	5 Pv/Pk (36) Pf/Pk (6) Pf/Pm (1) Total = 44	Pf/Pu/Pk (2) Pu/Pm/Pk (2)
m.	Calderaro et al. [4]	Italy (2000– 2007)	Febrile patients (701)	A		EDTA blood	High Pure Template Preparation kit (Roche Diagnostics, Mannheim, Germany)	185 rRNA	Nested PCR	159	Pf/Po (3) Pf/Pm (1) Total = 4	Pf/Pm/Po (2)

Table 1 Characteristics of the included studies

e 1 (continued) Author, Study a	Study a	area	Participants	Age range	Age groups	Blood	DNA	Investigated	PCR	Malaria	Mixed	Mixed
year (years of the survey)	(years of the survey)				(u)	storage for PCR	extraction	gene	method	positive by PCR	infection (dual infection)	infection (Triple infection)
Camargo- Colombia Febrile < Ayala (2012–2015) patients et al. [5] (671)	Colombia Febrile < (2012–2015) patients (671)	Febrile catients (671)	V	:5, 5–18, 18–60, and > 60 years	Triple infec- tion: < 5 (4), 5-18 (2), 18-60 (6), > 60 (6), > 60 (2) Dual infec- tion: < 5 (16), 5-18 (56), 18-60 (139), > 60	EDTA blood	Pure Link Genomic DNA mini kit (Invitrogen)	185 rRNA	Nested PCR	23	PF/Pm (190) PF/Pm (25) PF/Pm (10) Total = 225	Pf/Pm/Pv (14)
Dhangad- India (2008) Febrile and O- anajhi asympto- a et al. [6] matic (242)	India (2008) Febrile and 0– asympto- 6 matic (242)	Febrile and 0– asympto– matic (242)	0	5 years, 6–15, and > 15 years	Triple infec- tion: 0–5 (1), 6–15 (2), >15 (2), >15 (2) Dual infec- tion: 0–5 (7), 6–15 (32), >15 (32), >15	EDTA blood	Phenol–Chlo- roform extraction	185 rRNA	Nested PCR	19.	7 Pf/Pv (15) Pf/Pm (54) Pv/Pm (10) Total = 79	Pf/Pm/Pv (5)
Dormond Switzerland Malaria posi- >1 et al. [40] (2004–2008) tive (89)	Switzerland Malaria posi- >1 (2004–2008) tive (89)	Malaria posi- >1 tive (89)	$\overline{\wedge}$	6 years		EDTA blood	MagNA Pure LC DNA isolation kit (Roche, Basel, Swit- zerland)	185 rRNA	Nested PCR	õ	Pf/Po (3) Pf/Pm (2) Total = 5	Pf/Pm/Po (1)
Fuehrer Bangladesh: Febrile Any et al. [41] Chittagong patients Hill Tracts (379) (2007–2008) Malaria Research Initiative Bandarban field site (2008–2009)	Bangladesh: Febrile Any Chittagong patients Hill Tracts (379) (2007–2008) Malaria Research Initiative Bandarban field site (2008–2009)	Febrile Any patients (379)	Any	age /		Dried blood spots	Modified Chelex- based technique, the Insta- Gene Whole Blood Kit (Bio-Rad Laboratories, Hercules, CA)	185 rRNA	Nested PCR	18	 Pf/Pv (21) Pf/Pm (2) Total = 23 	Pf/Pm/Pv (2)

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No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups (n)	Blood storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
α	Gabrielli et al. [42]	Congo (2014)	Residents (306)	1 week to < 5 years		Dried blood spots	The com- mercial kit Dried Blood spot DNA isolation Kit (Norgen Biotek Corp, Ontario, Canada)	185 rRNA	Nested PCR		 4 Pf/Pm (13) Pf/Po (2) Pf/Pv (1) Total = 16 	Pf/Pm/Pv (1)
o.	Hopkins et al. [22]	Uganda (2010–2011)	Residents (272)	5-81 years: 5-10, 11-20, and ≥ 21 years		EDTA blood	Phenol–Chlo- roform extraction	185 rRNA	Nested PCR and qPCR	1	99 Pf/Pm (38) Pf/Po (8) Total = 46	Pf/Pm/Po (10)
10.	Jiang et al. [43]	Myanmar (2008)	Malaria posi- tive (146)	ЧA		Dried blood spots	Chelex extrac- tion kits	185 rRNA	Nested PCR	1~	46 Pf/Pk (13) Pv/Pk (13) Pf/Pv (10) Total = 36	Pf/Pv/Pk (2)
	Kasehagen et al. [11]	Papua New Guinea (2001–2003)	Residents (16,209)	< 2, 2.0-3.9, 4.0-6.9, 7.0-9.9, 10.0-19.9, 20.0-39.9, and \geq 40 years		EDTA blood	QlAamp 96 DNA Blood Kit (QlAGEN, Valencia, CA)	185 rRNA	LDR-FMA	59	 BPf/Pu (72) Pf/Pm (32) Pf/Pn (13) Pv/Pm (17) Pv/Po (9) Pm/Po (4) Total = 147 	Pf/Pm/Pv (28) Pf/Pv/Po (5) Pf/Pm/Po (4) Pv/Pm/Po (4) Pf/Pv/Pm/Po (11)
12.	Krishna et al. [18]	India (201 <i>5</i>)	Doubtful micro- scopic (355)	≤ 1, > 1-4, >4-8,>8-14, and>14 years		Dried blood spots	FavorPrep Genomic DNA Mini Kit (Favorgen Biotech Corp., Taiwan).	185 rRNA	Nested PCR		53 Pf/Pv (59) Pf/Pm (3) Total = 62	Pf/Pm/Pv (5) Pf/Pv/Po (1) Pf/Pv/Pm/ Po (1)
13.	Lorenzetti et al. [44]	Brazil (2003– 2005)	Malaria posi- tive (115)	18–52 years		EDTA blood	Phenol–Chlo- roform extraction	185 rRNA	Nested PCR	, _	5 Pf/Pm (2) Pf/Pv (28) Total = 30	Pf/Pm/Pv (1)
14.	Marques et al. [45]	Mozambique (2001–2002)	Residents (308)	1–82 years		EDTA blood	Phenol–Chlo- roform extraction	185 rRNA	Nested PCR	1	5 Pf/Pm (70) Pf/Po (10) Total = 80	Pf/Pm/Po (9)

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No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups (n)	Blood storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
15.	May et al. [48]	Nigeria (1996–1997)	Residents (593)	Children in Abanla (1–11 years), Children in Ibadan (3–8 years), Children in health centres in Ibadan (0.8– 11 years), Healthy adults (15–56 years)		EDTA blood	DNA-Easy Kit (Invitrogen, St. Louis, MO)	18S rRNA	Nested PCR	16	5 Pf/Pm (41) Pf/Po (12) Total = 53	Pf/Pm/Po (27)
16.	Mehlotra et al. [13]	Papua New Guinea (1998–1999)	Residents (1848)	All groups < 1–85 years: 2–4, 5–9, <u>></u> 10 years	Triple infection: $2-4$ tion: $2-4$ (2), $5-9$ (8), ≥ 10 (8), ≥ 10 (24) (24) Dual infection: $2-4$ tion: $2-4$ tion: $2-4$ (14), $5-9$ (51), ≥ 10 (115)	EDTA blood	Ol Aamp 96 spin blood kits (Ol AGEN, Valencia, CA)	185 rRNA	Nested PCR	Ϋ́Υ.	1 Pf/Pv (116) Pf/Pm (27) Pf/Pn (21) Pf/Po (11) Pv/Pm (21) Pv/Po (4) Pm/Po (3) Total = 182	Pf/Pm/Pv (23) Pf/Pv/Po (9) Pf/Pm/Po (1) P6/Pv/Pm/ Po (3)
17.	Mehlotra et al. [12]	Papua New Guinea (1996)	Malaria posi- tive (173)	5-10 and > 11 years	Triple infec- tion: 5–10 (1), >11 (0) Dual infec- tion: 5–10 (6), >11 (9)	EDTA blood	OlAamp 96 or individual spin blood kits (QIAGEN, Valencia, CA)	185 rRNA	Nested PCR	e –	3 Pf/Pv (40) Pf/Pm (16) Pf/Po (4) Pv/Pm (3) Total = 63	Pf/Pm/Pv (27) Pf/Pv/Po (8) Pf/Pm/Po (5) Pv/Pm/Po (1) Pf/Pv/Pm/ Po (9)
č	Mueller et al. [14]	Papua New Guinea (2005)	Residents (2527)	< 10 and ≥ 20 years		EDTA blood	OlAmp 96 DNA Blood kits (Qiagen, CA)	185 rRNA	A semi- quan- titative post-PCR, ligase detection/ fluo- rescent micro- sphere assay (LDR- FMA)		4 Pf/Pv (363) Pf/Pm (136) Pf/Po (27) Pv/Pm (26) Pv/Po (7) Pm/Po (1) Total = 560	Pf/Pm/Pv (99) Pf/Pv/Po (33) Pf/Pm/Po (6) Pf/Pv/Pm/Po (17)

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No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups (n)	Blood storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
19.	Nino et al. [46]	Colombia (2015–2016)	Febrile patients (1392)	ЧЧ		EDTA blood	Pure Link Genomic DNA mini kit (Invitrogen)	185 rRNA	Nested PCR	2.0	5 Pf/Pv (111) Pv/Pm (340) Pf/Pm (29) Total = 480	Pf/Pm/Pv (52)
20.	Pati et al. [19]	India	Febrile patients (1589)	Severe malaria (15–65 years)		Dried blood spots	Chelex extrac- tion kits	185 rRNA	Nested PCR	110) Pf/Pv (11) Pf/Pm (4) Pv/Pm (3) Total = 18	Pf/Pm/Pv (5)
21.	Perandin et al. [20]	Italy	Febrile patients (122)	A		EDTA blood	The High Pure PCR template preparation kit (Roche, Indianapolis, Ind.)	185 rRNA	Nested PCR	Ó	2 Pf/Po (1)	Pf/Pm/Po (1)
22.	Peruzzi et al. [21]	ltaly (2005– 2006)	Febrile patients (139)	2-49 years	Triple infection: 19 years	EDTA blood	High Pure PCR Template Preparation Kit"(Roche)	185 rRNA	Nested PCR	ñ	None	Pf/Pm/Po (1)
23.	Pong- vongsa et al. [28]	Laos-Vietnam border (2010)	Residents (3059) PCR (135)	P. knowlesi (2–15 years)	Triple infection (Pf/PV/ Pk): mean (3) years (7) Dual infec- tion (Pk/ PV): mean 7.4 years (5)	Dried blood spots	QlAamp DNA micro kit (QlAGEN, Tokyo, Japan)	185 rRNA	Nested PCR	δ	D Pf/PV (15) Pf/Pm (2 Pv/Pk (5) Total = 22	Pf/Pm/Pk (7) Pf/Pv/Pk (7)
24.	Putaporntip et al. [25]	Thailand (2006–2007)	Febrile patients (1874)	1-81 years		Dried blood spots	Qiagen DNA Mini Kit	185 rRNA	Nested PCR	175	Pf/Pv (200) Pf/Pm (6) Pf/Po (2) Pf/Pk (5) Pv/Pm (8) Pv/Pm (8) Pv/Pb (4) Pv/Pb (1) Pm/Po (1) Pm/Po (1)	Pf/Pm/Pv (4) Pf/Pv/Po (7) Pf/Pv/Pm/ Po (1)

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No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups (n)	Blood storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
25.	Rubio et al. [38]	Spain (1997– 1998)	Febrile patients (168)	₹ Z		EDTA blood	Modified Chelex- based technique, the Insta- Gene Whole Blood Kit (Bio-Rad Kit (Bio-Rad Hercules, CA)	18S rRNA	Nested PCR		89 Pf/Pm (4) Pf/Po (1) Pf/Pv (3) Total = 8	Pf/Pm/Pv (1)
26.	Rubio et al. [39]	Equatorial Guinea (1996)	Febrile patients (159)	< 6 years		Dried blood spots	5% Chelext-100 Resin (Bio- Rad Labora- tories, Hercules, CA)	185 rRNA	Nested PCR	F	26 Pf/Pm (36) Pf/Po (3) Pf/Pv (2) Total = 41	Pf/Pv/Po (3)
27.	Sitali et al. [29]	Zambia (2012)	Residents (873)	< 6 years		Dried blood spots	Chelex extrac- tion kits	185 rRNA	Nested PCR	4	74 Pf/Pm (31) Pf/Po (10) Pf/Pv (1) Total = 42	Pf/Pm/Po (6) Pf/Pm/Pv (1)
28.	Sluydts et al. [15]	. Cambodia (2012)	Residents (5793)	2–5, 5–14, 15–39, and ≥ 40 years		Dried blood spots	Instagene [®] Matrix resin (Bio-Rad, Singapore)	185 rRNA	Two-step Real-time PCR	ñ	 Pf/Pv (56) Pf/Pm (5) Pf/Po (1) Pv/Pm (3) Pv/Po (11) Total = 76 	Pf/Pm/Pv (4) Pf/Pv/Po (1) Pf/Pv/Pm/ Po (1)
29.	Steenkeste et al. [16]	Cambodia (2001)	Residents (337)	۲		Dried blood spots	the Instagene resin (Bio- Rad, USA)	185 rRNA	Nested PCR	È	40 Pf/Pv (52) Pf/Pm (15) Pf/Po (4) Total = 71	Pf/Pu/Po (7) Pf/Pm/Pv (24) Pf/Pv/Pm/ Po (8)
30.	Steenkeste et al. [17]	Cambodia (2001)	Residents (134)	< 1-> 60 years: <1, 1, 2, 3, 4, 5-9, 10-14, 15-19, 20-39, 40-60, and > 60 years		Dried blood spots	Instagene resin (Bio-Rad, Germany) a	18S rRNA	Nested PCR	-	22 Pf/Pm (4) Pf/Po (2) Pf/Pv (18) Pv/Pm (3) Total = 27	Pf/Pm/Pv (7) Pf/Pv/Po (3) Pf/Pv/Pm/ Po (3)

ar	Ne I (contir	(panu										
No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups (n)	Blood storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
31.	Subissi et al. [23]	Uganda (2010)	Residents (509)	1-5, 6-10, and > 20 years	Triple infec- (5), 6-10 (5), 20 (0) (0) Dual infec- tion: 1–5 (27), 6-10 (24), > 20 (2)	Dried blood spots	QlAamp mini kit (QIAGEN, Venlo, The Nether- Iands)	18S FRNA	Nested PCR	7	99 Pf/Pm (39) Pf/Po (14) Total = 53	Pf//Pm/Po (10)
32.	Toma et al. [27]	Laos (1997)	Residents (336)	 < 11- > 50 years: 2-68 years in Phavang and 0-75 years in Sisom-souen 		EDTA blood	GFX Genomic Blood DNA Purification Kit (Pharmacia Biotech).	18S rRNA	Nested PCR	-	17 Pf/Pv (19) Pf/Pm (2) Pv/Pm (2) Pv/Po (1) Total = 24	Pf/Pm/Pv (1) Pf/Pv/Po (1) Pf/Pv/Pm/ Po (1)
33.	Woldear- egai et al. [47]	Gabon (2016)	Residents (834)	1–96 years: 1–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–40, 41–50, 51–60, 61–70, 71–80, and 81–96 years		EDTA blood	QlAsymphony DSP DNA kit	185 rRNA	Nested PCR	U	.18 Pf/Pm (123) Pf/Po (43) Pm/Po (1) Total = 167	Pf//Pm/Po (51)
34.	Zhou et al. [24]	Thailand (1995–1996)	Residents (548)	A		Thick smear	30% Chelex-100	185 rRNA	Nested PCR	-	14 Pf/Pv (10) Pf/Pm (6) Pv/Pm (19) Pv/Po (1) Total = 36	Pf/Pm/Pv (49) Pf/Pv/Po (2) Pv/Pm/Po (1) Pf/Pv/Pm/ Po (7)
35.	Zhou et al. [26]	China (2008– 2012)	Febrile patients (562)	A		Dried blood spots	QlAamp DNA Mini Kit (QlAGEN China (Shanghai)	185 rRNA	Nested PCR	m	84 Pf/Pv (67) Pv/Po (2) Pf/Pk (2) Pf/Pm (2) Po/Pm (1) Total = 74	Pf/Pv/Po (3) Pf/Pm/Pv (1) Pv/Pm/Po (1) Pf/Pv/Pm/ Po (1)

No.	Author, year	Study area (years of the survey)	Participants	Age range	Age groups Blood (n) storage for PCR	DNA extraction	Investigated gene	PCR method	Malaria positive by PCR	Mixed infection (dual infection)	Mixed infection (Triple infection)
									Total = 12,023	2 spe-	3 spe-
										cies = 3059	cies = 601
										Pf/Pv = 1318	Pf/Pm/
										Pf/Po = 188	Pv = 355
										Pf/Pm = 775	Pf/Pv/Po = 83
										Pf/Pk = 26	Pf/Pm/
										Pv/Pm = 645	Po = 137
										Pv/Po = 39	Pf/Pv/Pk = 11
										Pv/Pk = 58	Pv/Pm/
										Pm/Po = 10	Po = 13
										Pm/Pk = 1	Pv/Pm/Pk=2
											4 species = 53
Pf refe	ers to P. falcipa	"mm									

Table 1 (continued)

v refers to <i>P. vivax</i> o refers to <i>P. ovale</i> m refers to <i>P. malariae</i> k refers to <i>P. knowlesi</i> VA Not Assessed	Pf refers to P. falciparum
o refers to <i>P. ovale</i> m refers to <i>P. malariae</i> k refers to <i>P. knowlesi</i> A Not Assessed	'v refers to <i>P. vivax</i>
m refers to <i>P. malariae</i> k refers to <i>P. knowlesi</i> /A Not Assessed	o refers to <i>P. ovale</i>
k refers to <i>P. knowlesi</i> /A Not Assessed	m refers to <i>P. malariae</i>
IA Not Assessed	k refers to <i>P. knowlesi</i>
	IA Not Assessed

										-
No.	Kererences	Selection				Compatibility	Exposure			fotal
		ls the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls		Ascertainment of exposure	Same method of ascertainment for cases and controls	Non- response Rate	(2)
_:	Asua et al. [2]	*			NA	*	*	*	*	5
2	Barber et al. [3]	*			NA	*	*	*	*	5
ю.	Calderaro et al. [4]	*	*		NA	*	*	*	*	9
4.	Camargo-Ayala et al. [5]	*	*		NA	*	*	*	*	9
5.	Dhangadamajhi et al. [6]	*	*		NA	*	*	*	*	9
õ.	Dormond et al. [40]	*			NA	*	*	*	*	5
7.	Fuehrer et al. [41]	*	*		NA	*	*	*	*	9
¢.	Gabrielli et al. [42]	*	*	*	NA	*	*	*	*	7
9.	Hopkins et al. [22]	*			NA	*	*	*	*	7
10.	Jiang et al. [43]	*			NA	*	*	*	*	2
11.	Kasehagen et al. [11]	*	*	*	NA	*	*	*	*	7
12.	Krishna et al. [18]	*			NA	*	*	*	*	5
13.	Lorenzetti et al. [44]	*			NA	*	*	*	*	5
4.	Marques et al. [45]	*	*	*	NA	*	*	*	*	7
15.	May et al. [7]	*	*	*	NA	*	*	*	*	7
16.	Mehlotra et al. [13]	*	*	*	NA	*	*	*	*	7
17.	Mehlotra et al. [12]	*			NA	*	*	*	*	5
<u>1</u> 8	Mueller et al. [14]	*	*	*	AN	*	*	*	*	7
19.	Nino et al. [46]	*	*		NA	*	*	*	*	9
20.	Pati et al. [19]	*	*		NA	*	*	*	*	9
21.	Perandin et al. [20]	*	*		NA	*	*	*	*	9
22.	Peruzzi et al. [21]	*	*		NA	*	*	*	*	9
23.	Pongvongsa et al. [28]	*	*	*	NA	*	*	*	*	7
24.	Putaporntip et al. [25]	*	*		NA	*	*	*	*	9
25.	Rubio et al. [38]	*	*		NA	*	*	*	*	9
26.	Rubio et al. [38]	*	*		NA	*	*	*	*	9
27.	Sitali et al. [29]	*	*	*	NA	*	*	*	*	7
28.	Sluydts et al. [15]	*	*	*	NA	*	*	*	*	7
29.	Steenkeste et al. [16]	*	*	*	NA	*	*	*	*	7
30.	Steenkeste et al. [17]	*	*	*	NA	*	*	*	*	7
31.	Subissi et al. [23]		*	*	NA	*	*	*	*	7
32.	Toma et al. [<mark>27</mark>]		*	*	NA	*	*	*	*	7
33.	Woldearegai et al. [47]	*	*	*	NA	*	*	*	*	7

Table 2 Quality of the included studies

No.	References	Selection				Compatibility	Exposure			Total
		ls the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls		Ascertainment of exposure	Same method of ascertainment for cases and controls	Non- response Rate	score (7)
34.	Zhou et al. [24]	*	*	*	NA	*	*	*	*	7
35.	Zhou et al. [<mark>26</mark>]	*	*		NA	*	*	*	*	9



13% (95% CI 8–21%) and dried blood spots was 10% (95% CI 7–17%), with no subgroup difference between the groups (P-value = 0.59; Fig. 6).

Seven included studies reported the age groups, and different types of mixed infection (85 triple mixed infection and 581 double mixed infection). Subgroup analysis of age groups demonstrated that, compared with the proportion of double mixed infection, triple mixed infection was lower in patients aged \leq 5 years (OR=0.27; 95% CI 0.13–0.56; I²=31%) and >5 years (OR=0.09; 95% CI 0.04–0.25; I²=78%) (Fig. 7). Subgroup analysis demonstrated no statistical difference (P-value=0.09, I²=64.7%).

Publication bias

Publication bias related to study effects was assessed using funnel plot asymmetry, and no publication bias was demonstrated as evidenced by the symmetry of the funnel plot (Fig. 8).

Discussion

The pooled prevalence of triple mixed infection has not been clarified in the previous literature. The systematic review and meta-analysis of 12,023 malaria-positive patients demonstrated a high prevalence of triple mixed infection (4%). The most common triple mixed infection of *Plasmodium* species was *P. falciparum, P. malariae*, and *P. vivax* (59%). This finding agreed with those of previous studies in Papua New Guinea [11– 14], India [18], Cambodia [15–17], and Thailand [24], but contradicts the findings of previous studies which included *P. ovale* in triple mixed infection in the Laos-Vietnam border (*P. falciparum/P. vivax/P. knowlesi*) [28], China (*P. falciparum/P. vivax/P. ovale*) [26], Thailand (*P. falciparum/P. vivax/P. ovale*) [25], and Zambia (*P. falciparum/P. malariae/P. ovale*) [29].

The mechanisms underlying the triple mixed parasitic infection are unknown; however, the course of an infection might be influenced by the simultaneous occurrence

	Triple infe	ctions	Dual infe	ctions		Odds Ratio	Odds Ratio	Risk of Bias
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 9	5% CI A B C D E F G
Asua et al., 2017	1	474	37	474	1.9%	0.02 [0.00, 0.18]	←	
Barber et al., 2012	4	445	44	445	3.0%	0.08 (0.03, 0.23)		
Calderaro et al., 2008	2	159	4	159	2.1%	0.49 (0.09, 2.73)		
Camargo-Ayala et al., 2016	14	531	225	531	3.5%	0.04 [0.02, 0.06]	_ _	
Dhangadamajhi et al., 2009	5	197	79	197	3.1%	0.04 [0.02, 0.10]		
Dormond et al., 2010	1	89	5	89	1.7%	0.19 [0.02, 1.67]		
Fuehrer et al., 2010	2	189	23	189	2.4%	0.08 [0.02, 0.33]		
Gabrielli et al., 2016	1	164	16	164	1.8%	0.06 [0.01, 0.43]		
Hopkins et al., 2013	10	199	46	199	3.3%	0.18 (0.09, 0.36)	<u> </u>	
Jiang et al., 2010	2	146	36	146	2.4%	0.04 [0.01, 0.18]		
Kasehagen et al., 2006	41	658	147	658	3.7%	0.23 [0.16, 0.33]		
Krishna et al., 2017	6	353	62	353	3.2%	0.08 (0.03, 0.19)	<u> </u>	
Lorenzetti et al., 2008	1	115	30	115	1.8%	0.02 (0.00, 0.19)	←	
Marques et al., 2005	9	115	80	115	3.3%	0.04 [0.02, 0.08]	_ _	
May et al., 1999	27	165	53	165	3.5%	0.41 [0.24, 0.70]		
Mehlotra et al., 2000	41	163	63	163	3.6%	0.53 [0.33, 0.86]		
Mehlotra et al., 2002	33	541	182	541	3.6%	0.13 [0.09, 0.19]	-	
Mueller et al., 2009	146	1844	560	1844	3.8%	0.20 [0.16, 0.24]	-	
Niño et al., 2016	52	596	480	596	3.7%	0.02 [0.02, 0.03]	-	
Pati et al., 2017	5	110	18	110	3.0%	0.24 [0.09, 0.68]		
Perandin et al., 2004	1	62	1	62	1.2%	1.00 (0.06, 16.35)		
Pongvongsa et al., 2018	8	90	22	90	3.2%	0.30 (0.13, 0.72)	_	
Putaporntip et al., 2009	11	1751	230	1751	3.5%	0.04 (0.02, 0.08)	_ - _	
Rubio et al. (2), 1999	1	89	8	89	1.7%	0.12 (0.01, 0.94)		
Rubio et al., 1999	3	126	41	126	2.7%	0.05 (0.02, 0.17)		
Sitali et al., 2015	7	474	42	474	3.2%	0.15 (0.07, 0.35)		
Sluvdts et al., 2014	5	368	76	368	3.1%	0.05 (0.02, 0.13)		
Steenkeste et al., 2009	31	140	71	140	3.5%	0.28 (0.16, 0.46)	_ _	
Steenkeste et al., 2010	10	102	27	102	3.3%	0.30 (0.14, 0.66)		
Subissi et al 2019	10	299	53	299	3.4%			
Toma et al. 2001	2	117	24	117	2.4%			
Woldearenai et al 2019	51	618	167	618	3.7%	0.24 (0.17 0.34)		
Zhou et al., 1998	52	114	36	114	3.5%	1.82 [1.06.3.12]	⊢ ⊷	
Zhou et al., 2014	5	384	74	384	3.1%	0.06 [0.02, 0.14]		
Total (95% CI)		11987		11987	100.0%	0.12 [0.09, 0.18]	•	
Total events	600		3062					
Heterogeneity: Tau ² = 0.98° CF	ni ² = 365.34	df = 33 (P < 0.0000	1): P = 91	1%			
Test for overall effect: $7 = 10.7$	9 (P < 0 000	011)	0.0000	.,,. = 0	• • •	_	0.01 0.1 1	10 100
	5 th - 0.000	.,				F	avours [experimental] Favo	urs [control]

of several Plasmodium species [30, 31]. Another explanation is the immunosuppressive effects caused by chronic P. falciparum infection and differences in individual exposure [32]. Whether the simultaneous infections might be beneficial or adds further detriment to the infected individual is not well defined. Triple mixed infection may be caused by cross immunity-induced susceptibility to three infections or exposure to infective bites of a single vector that can transmit three *Plas*modium species [33]. A previous study indicated that infection with one Plasmodium species increased susceptibility to infection by other *Plasmodium* species [34]. The apparent frequency of mixed infection is dependent on the technique used for parasite analyses. The results demonstrated a high proportion of triple mixed infection compared with double mixed infection only in studies using PCR analysis to detect the malaria parasite due to the high sensitivity and specificity of PCR compared with microscopy or RDTs. In areas where more than one *Plasmodium* species is present and transmission is stable, the adult populations often have parasite densities below the level of microscopic detection and called "submicroscopic infections". These submicroscopic infections demonstrated more than one *Plasmodium* species.

The subgroup analysis demonstrated that the proportion of triple mixed infection was higher in residents than in febrile patients, indicating that residents in communities where malaria is endemic were exposed to malaria several times or to more than one species at a time [35]. These triple mixed infection were submicroscopic infection for which microscopy has insufficient sensitivity for their detection. It is well-documented that malaria patients in endemic areas develop immunity against malaria, resulting in symptom relief [35, 36]. A previous study demonstrated that age, geographical origin, and clinical manifestations were found to be associated with triple mixed infection [5]. The subgroup analysis of age ranges demonstrated that the proportion of triple mixed infection was significantly lower across a wide age range of ages compared to double mixed infection. Subgroup analysis demonstrated that no statistical difference in age groups and types of mixed infection. This result suggested that triple mixed infection can occur in both patients aged \leq 5 years and > 5 years. However, a limited

	Triple infections Dual infections		Odds Ratio		Odds Ratio		
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl
1.2.1 Asia							
Barber et al., 2012	4	445	44	445	3.0%	0.08 (0.03, 0.23)	
Dhangadamajhi et al., 2009	5	197	79	197	3.1%	0.04 (0.02, 0.10)	
Fuehrer et al., 2010	2	189	23	189	2.4%	0.08 [0.02, 0.33]	
Jiang et al., 2010	2	146	36	146	2.4%	0.04 [0.01, 0.18]	
Krishna et al., 2017	6	353	62	353	3.2%	0.08 [0.03, 0.19]	
Pati et al., 2017	5	110	18	110	3.0%	0.24 [0.09, 0.68]	
Pongvongsa et al., 2018	8	90	22	90	3.2%	0.30 [0.13, 0.72]	
Putaporntip et al., 2009	11	1751	230	1751	3.5%	0.04 [0.02, 0.08]	
Sluydts et al., 2014	5	308	70 74	368	3.1%	0.05 [0.02, 0.13]	
Steenkeste et al., 2009 Steenkeste et al., 2009	31	140	27	140	3.5%	0.28 [0.16, 0.46]	
Tomo et al. 2001	10	102	27	102	3.370		
7 bou et al., 2001	5	204	24	204	2.4%		
Zhou et al., 1990 Zhou et al. 1999	52	11/	26	114	2.5%	1 92 [1 06 3 12]	
Subtotal (95% Cl)	52	4506	30	4506	42.7%	0.12 [0.06, 0.25]	
Total events	1/18		977		1211 10	0112 [0100, 0120]	•
Heterogeneity: Tau ² = 1.77; Ch Test for overall effect: Z = 5.58	i ² = 140.72, ((P < 0.00001	df = 13 (i)	P < 0.0000	1); I² = 91	%		
1.2.2 Africa							
Asua et al., 2017	1	474	37	474	1.9%	0.02 (0.00, 0.18)	←
Gabrielli et al., 2016	1	164	16	164	1.8%	0.06 [0.01, 0.43]	
Hopkins et al., 2013	10	199	46	199	3.3%	0.18 (0.09, 0.36)	
Marques et al., 2005	9	115	80	115	3.3%	0.04 [0.02, 0.08]	
May et al., 1999	27	165	53	165	3.5%	0.41 [0.24, 0.70]	
Rubio et al. (2), 1999	1	89	8	89	1.7%	0.12 [0.01, 0.94]	
Sitali et al., 2015	7	474	42	474	3.2%	0.15 [0.07, 0.35]	
Subissi et al., 2019	10	299	53	299	3.4%	0.16 [0.08, 0.32]	
Woldearegai et al., 2019 Subtotal (95% CI)	51	618 2597	167	618 2597	3.7% 25.8 %	0.24 [0.17, 0.34] 0.14 [0.08, 0.24]	▲
Total events	117		502				
Heterogeneity: Tau ² = 0.44; Ch Test for overall effect: Z = 7.05	ii² = 33.65, df (P < 0.00001	′=8(P∝)	< 0.0001); I ^a	² = 76%			
1.2.3 America							
Camargo-Ayala et al., 2016	14	531	225	531	3.5%	0.04 [0.02, 0.06]	
Lorenzetti et al., 2008	1	115	30	115	1.8%	0.02 [0.00, 0.19]	•
Niño et al., 2016	52	596	480	596	3.7%	0.02 [0.02, 0.03]	
		1242	705	1242	9.0%	0.03 [0.02, 0.04]	•
I otal events	b/	a (n	735	~			
Test for overall effect: Z = 23.91	1 (P < 0.0000	= 2 (P = 1)1)	0.36); 1* = 1	70			
1.2.4 Europe							
Calderaro et al., 2008	2	159	4	159	2.1%	0.49 [0.09, 2.73]	
Dormond et al., 2010	1	89	5	89	1.7%	0.19 [0.02, 1.67]	
Perandin et al., 2004	1	62	1	62	1.2%	1.00 [0.06, 16.35]	
Rubio et al., 1999	3	126	41	126	2.7%	0.05 [0.02, 0.17]	
Subtotal (95% CI)	_	436		436	7.8%	0.21 [0.05, 0.86]	
Total events Heterogeneity: Tau² = 1.17; Ch Test for overall effect: Z = 2.17	7 ii² = 7.02, df = (P = 0.03)	= 3 (P = 1	51 0.07); I² = 5	7%			
1.2.5 Oceania							
Kasehagen et al., 2006	41	658	147	658	3.7%	0.23 (0.16. 0.33)	
Mehlotra et al., 2000	41	163	63	163	3.6%	0.53 (0.33, 0.86)	
Mehlotra et al., 2002	33	541	182	541	3.6%	0.13 [0.09, 0.19]	—
Mueller et al., 2009	146	1844	560	1844	3.8%	0.20 [0.16, 0.24]	-
Subtotal (95% CI)		3206		3206	14.7%	0.23 [0.15, 0.36]	◆
Total events Heterogeneity: Tau ² = 0.18; Ch Test for overall effect: Z = 6.29	261 ii² = 21.58, df (P < 0.00001	⁷ =3(P ∝)	952 0.0001); I ^a	² = 86%			
Total (95% CI)		11987		11987	100.0%	0.12 [0.09, 0.18]	◆
Total events	600		3062				
Heterogeneity: Tau ² = 0.98; Ch	ii [≈] = 365.34, (a (P < 0 0000	df = 33 (i 11)	P < 0.0000 [.]	1); I² = 91	%		
Test for subgroun differences:	Chi ² = 77 54	. df = 4 /	°P < 0 0000	1), I ² = 9	4.8%		Favours [experimental] Favours [control]
Fig. 4 Subgroup analysis of tr	riple mixed	infectio	ons by Pla	smodiur	n specie	es between areas of th	ne included studies

Study of singroup Events Total Weight M-H, Random, 95% CI M-H, Random, 95% CI A B C D E F G Calderare tal, 2006 2 159 4 169 2.7% 0.49 (0.09, 2.73) Calderare tal, 2010 1 631 1225 531 4.4% 0.004 (0.02, 0.03) Fuehrer tal, 2010 2 158 1225 531 4.4% 0.040 (0.02, 0.03) Prandin etal, 2001 5 110 18 110 3.7% 0.24 (0.09, 0.68) Prandin etal, 2009 11 1751 4.4% 0.04 (0.02, 0.06)		Triple infections		Dual infections		Odds Ratio		Odds Ratio		Risk of Bias	
1.3.1 Febrile Calderato et al., 2008 2 159 4 159 2.7% 0.49 (0.09, 2.73) Camargo-Ayala et al., 2016 14 531 2.25 531 4.4% 0.04 (0.02, 0.03) Niño et al., 2016 52 566 480 596 4.6% 0.02 (0.02, 0.03) Peradin et al., 2004 1 62 1 62 1.5% 1.00 (0.06, 10.35) Prendin et al., 2004 1 62 1 62 1.5% 1.00 (0.06, 10.35) Puisoperije et al., 2019 1 162 1.4% 0.04 (0.02, 0.08) ++++++++++++++++++++++++++++++++++++	Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Randor	n, 95% Cl	ABCDEFG	
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Camargo-Ayala et al., 2016 14 631 225 531 4.4% 0.04 (0.02, 0.06) + Puther et al., 2016 52 696 480 596 4.8% 0.02 (0.02, 0.03) + Paral et al., 2017 5 110 18 110 3.7% 0.24 (0.03, 0.08) + Parantin et al., 2004 1 62 15% 1.00 [0.05, 16.35] + Puthor et al., 2019 1 89 8 99 2.2% 0.12 (0.01, 0.94] + Puthor et al., 2014 5 384 74 384 3.9% 0.06 [0.02, 0.14] + Puthor et al., 2014 5 384 74 384 3.9% 0.06 [0.02, 0.14] + Parantin et al., 2014 5 384 74 384 3.9% 0.06 [0.02, 0.14] + Parantin et al., 2014 5 384 74 384 3.9% 0.06 [0.02, 0.14] + Parantin et al., 2015 1 1 164 16 164 2.3% 0.06 [0.02, 0.14] + Parantin et al., 2016 1 1 164 16 164 2.3% 0.06 [0.02, 0.14] + Parantin et al., 2016 1 1 164 16 164 2.3% 0.06 [0.02, 0.03] + Hetrogonelity. Tau ⁺ 0.65 (C). The 30P 7 3399 7 34.0% 0.07 [0.04, 0.13] + Parantin et al., 2015 1 1 164 16 164 2.3% 0.06 [0.02, 0.03] + Hetrogonelity. Tau ⁺ 0.5 (C). The 37.40, df = 9 (P < 0.0001); P 76%. Test for overall effect Z = 8.78 (P < 0.00001) = 76%. Test for overall effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.78 (P < 0.00001); P 76%. Test for averal effect Z = 8.76 (P < 0.00001); P 76%. Test for averal effect Z = 8.76 (P < 0.00001); P 86%. Test for averal effect Z = 8.67 (P < 0.00001); P 86%. Test for averal effect Z = 8.67 (P < 0.00001); P 86%. Test for averal effect Z = 8.16 (P < 0.00001); P 86%. Test for averal effect Z = 8.16 (P < 0.00001); P 86%. Test for overall effect Z = 8.16 (P < 0.00001); P 86%. Test for overall effect Z = 8.16 (P < 0.00001); P 86%. Test for overall effect Z = 8.16 (P < 0.00001); P 86%. Test for averal effect Z = 8.16 (P < 0.00001); P 86%. Test for averal effect Z = 8.16 (P < 0.00001);	Calderaro et al., 2008	2	159	4	159	2.7%	0.49 [0.09, 2.73]				
Fuehrer et al., 2010 2 189 3.23 189 3.0% 0.08 [0.02, 0.3]	Camargo-Ayala et al., 2016	14	531	225	531	4.4%	0.04 (0.02, 0.06)				
Niñe stal, 2016 52 596 480 596 4.6% 0.02 [0.02, 0.03] + Perantin et al., 2017 5 110 18 110 3.7% 0.24 [0.09, 0.68] + Prantin et al., 2004 1 62 1 62 1.5% 1.00 [0.06, 16.35] Putaonitio et al., 2019 11 1751 230 1751 4.4% 0.04 [0.02, 0.08] + Putaonitio et al., 2019 12 21 128 4.41 128 3.4% 0.06 [0.02, 0.17] + Puto et al., 1999 3 126 41 128 3.4% 0.06 [0.02, 0.17] + Puto et al., 1999 3 126 41 128 3.4% 0.06 [0.02, 0.17] + Puto et al., 2014 5 3264 774 3264 3.9% 0.06 [0.02, 0.17] + Puto et al., 2015 1 164 166 164 2.3% 0.06 [0.01, 0.43] + Heterogeneity, Tau ² = 0.56; Ch ² = 37.40; df = 9 ($P = 0.0001$); $P = 76\%$ Total events 96 1104 Heterogeneity Tau ² = 0.56; Ch ² = 37.40; df = 9 ($P = 0.0001$); $P = 76\%$ Heterogeneity Tau ² = 0.15; Ch ² = 37.40; df = 9 ($P = 0.0001$); $P = 76\%$ Stati et al., 2016 1 164 16 164 2.3% 0.06 [0.01, 0.43] + Heterogeneity, Tau ² = 0.15; Ch ² = 37.40; df = 9 ($P = 0.0001$); $P = 76\%$ Heterogeneity Tau ² = 0.15; Ch ² = 37.40; df = 9 ($P = 0.0001$); $P = 76\%$ Stati et al., 2005 41 658 147 658 4.6% 0.23 [0.16, 0.33] + Heterogeneity, Tau ² = 0.42; Ch ² = 11.18; df = 156 4.45\% 0.18 [0.00, 0.19] + Heterogeneity, Tau ² = 0.42; Ch ² = 11.14 36 114 4.5\% 0.28 [0.16, 0.24] + Promyongs et al., 2019 10 229 00 4.0% 0.30 [0.13, 0.72] + Stati et al., 2016 7 474 42 474 4.1% 0.15 [0.07, 0.35] + Heterogeneity, Tau ² = 0.42; Ch ² = 11.16; df = 15 ($P < 0.00001$); $P = 86\%$ Steenkeste et al., 2010 10 102 27 102 4.1% 0.38 [0.16, 0.24] + Promyongs et al., 2019 10 229 53 229 4.2% 0.16 [0.08, 0.22] + Total events 4.3 1602 Heterogeneity, Tau ² = 0.42; Ch ² = 11.01; df = 15 ($P < 0.00001$); $P = 86\%$ Test for overall effect Z = 8.16 ($P < 0.00001$); $P = 86\%$ Test for overall effect Z = 8.16 ($P < 0.00001$); $P = 82.5\%$ Fig. 5 Subgroup analysis of triple mixed infections by Plasmadium species between residents and febrile groups	Fuehrer et al., 2010	2	189	23	189	3.0%	0.08 (0.02, 0.33)				
Paid et al., 2017 5 110 18 110 3.7% 0.24 $[0.09, 0.68]$ Prenandin et al., 2009 11 1751 220 1751 4.4% 0.04 $[0.02, 0.08]$ Pubio et al., 1999 3 128 41 128 3.4% 0.05 $[0.02, 0.17]$ Pubio et al., 1999 3 128 41 128 3.4% 0.06 $[0.02, 0.17]$ Pubio et al., 1999 3 128 41 128 3.4% 0.06 $[0.02, 0.17]$ Pubio et al., 2014 5 384 74 384 3.9% 0.06 $[0.02, 0.17]$ Total events 96 1104 Heterogeneity, Tau"= 0.56; Ch ²⁺ 37.40, df = 9 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 8.78 (P < 0.0001); P = 76% Test for overall effect. Z = 0.10 41 658 147 658 4.6% 0.23 [0.16, 0.33] \rightarrow Hendriar et al., 2006 41 658 147 658 4.6% 0.23 [0.16, 0.33] \rightarrow Hendriar et al., 2006 146 1844 4.8% 0.21 [0.16, 0.31] \rightarrow Hendriar et al., 2009 146 1844 4.8% 0.21 [0.16, 0.31] \rightarrow Mueller et al., 2009 146 1844 4.8% 0.21 [0.16, 0.24] $+$ Pongovings et al., 2014 5 386 76 388 3.9% 0.05 [0.02, 0.13] \rightarrow Steenkeste et al., 2011 10 102 27 102 4.1% 0.33 [0.14, 0.66] \rightarrow Steenkeste et al., 2010 10 102 27 102 4.1% 0.32 [0.16, 0.46] \rightarrow Steenkeste et al., 2010 10 102 27 102 4.1% 0.32 [0.16, 0.46] \rightarrow Total (95% Ch) 60008 60008 66.0% 0.24 [0.17, 0.34] $+$ Pondovings et al., 2019 51 618 167 6 18 4.7% 0.24 [0.17, 0.34] $+$ Total (95% Ch) 60008 60008 66.0% 0.24 [0.17, 0.34] $+$ Total (95% Ch) 60008 60008 66.0% 0.24 [0.17, 0.34] $+$ Total (95% Ch) 10005 100.0% 0.14 [0.09, 0.21] \bullet Total (95% Ch) 60008 60008 66.0% 0.24 [0.17, 0.34] $+$ Heterogeneity, Tau"= 0.98; Ch ² = 31.509, (f = 25, P < 0.00001); P = 92% Test for overall effect. Z = 8.16 (P < 0.00001); P = 92% Test for overall effect. Z = 8.16 (P < 0.00001); P = 92% Test for overall effect. Z	Niño et al., 2016	52	596	480	596	4.6%	0.02 (0.02, 0.03)	-			
Perandin et al., 2004 1 62 1 62 1.5% 1.00 [0.05, 16.35] Putaporting te al., 2009 11 1751 2.4% 0.04 [0.02, 0.06] Publo et al. (2), 1999 1 83 8 89 2.2% 0.12 [0.01, 0.94] Publo et al. (2), 1999 1 83 26 41 126 3.4% 0.05 [0.02, 0.17] Zhou et al., 2014 5 384 74 384 3.9% 0.06 [0.02, 0.14] Subtotal (9% C) 3997 3997 34.0% 0.07 [0.04, 0.13] Total events 9 6 1104 Heterogeneity. Tau ² = 0.56; Ch ² = 37.40, df = 9 ($P < 0.0001$); P= 76% Test for overal effect Z = 8.78 ($P < 0.00001$) 1.3.2 Residents Gabrielli et al., 2016 1 164 16 199 4.2% 0.18 [0.09, 0.36] Harques et al., 2006 41 668 147 658 4.6% 0.23 [0.16, 0.33] Harques et al., 2006 41 668 147 658 4.6% 0.23 [0.16, 0.33] Herbotrs et al., 2006 11 564 146 580 1154 4.1% 0.04 [0.02, 0.08] Harques et al., 2006 14 658 147 658 4.6% 0.23 [0.16, 0.33] Harques et al., 2006 14 658 147 658 4.6% 0.23 [0.16, 0.33] Harques et al., 2006 14 658 147 658 4.6% 0.23 [0.16, 0.33] Harques et al., 2006 14 658 147 658 4.6% 0.23 [0.16, 0.33] Harques et al., 2001 14 618 44 8.5% 0.20 [0.16, 0.24] Hueller et al., 2003 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] Hueller et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] Hueller et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] Hueller et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] Hueller et al., 2009 11 140 71 140 4.5% 0.28 [0.16, 0.46] Heterogeneity. Tau ² = 0.42; Ch ² = 1.01.18, df = 15 ($P < 0.00001$); $P = 86\%$ Steenkeste et al., 2019 10 229 53 229 4.2% 0.16 [0.08, 0.52] Total events 4.3 1602 Heterogeneity. Tau ² = 0.42; Ch ² = 110.18, df = 15 ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Test for overal effect $Z = 9.16$ ($P < 0.00001$); $P = 86\%$ Tes	Pati et al., 2017	5	110	18	110	3.7%	0.24 (0.09, 0.68)				
Putapornip et al. 2009 11 1751 230 1751 4.4% 0.04 ($0.2, 0.08$) ++ Putable et al. (2199 3 126 41 126 3.4% 0.05 ($0.02, 0.13$) ++ Putable et al. (2014 5 334 74 384 339, 0.06 ($0.02, 0.14$) ++ Putable et al. (2014 5 334 74 384 339, 0.06 ($0.02, 0.14$) ++ Putable et al. (2014 5 334 74 384 339, 0.06 ($0.02, 0.14$) ++ Putable et al. (2016 1 164 16 164 2.3% 0.06 ($0.01, 0.43$) ++ Heterogeneity. Tau" = 0.56; Chi ^m = 37.40, df = 9 ($P < 0.00001$); P= 76%. Test for overall effect $Z = 8.78 (P < 0.00001)$ 1.3.2 Residents Cobriell et al. 2006 41 658 147 658 45% 0.23 ($1.6, 0.33$) ++ Hehorize et al. 2006 41 658 147 658 45% 0.23 ($1.6, 0.33$) ++ Marques et al. 2006 41 658 147 658 45% 0.23 ($1.6, 0.33$) ++ Hehorize et al. 2006 41 658 147 658 45% 0.23 ($1.6, 0.33$) ++ Hehorize et al. 2006 41 658 147 658 45% 0.23 ($1.6, 0.33$) ++ Hehorize et al. 2006 41 658 147 658 45% 0.23 ($1.6, 0.33$) ++ Hehorize et al. 2005 9 115 80 115 4.1% 0.04 ($0.02, 0.08$) ++ Hehorize et al. 2009 146 1844 45% 0.31 ($0.02, 0.19$) ++ Hehorize et al. 2014 5 368 76 388 3.005 ($0.02, 0.13$) ++ Hehorize et al. 2015 7 474 42 474 4.1% 0.15 ($0.07, 0.35$] ++ Hehorize et al. 2010 10 10 2 27 102 4.1% 0.30 ($1.6, 0.32$) ++ Steant et al. 2010 10 102 27 102 4.1% 0.30 ($1.4, 0.58$] ++ Steenkeste et al. 2010 10 102 27 102 4.1% 0.30 ($1.4, 0.58$] ++ Steant et al. 2010 12 117 24 117 3.0% 0.07 ($0.22, 0.23$] ++ Heterogeneity. Tau" = 0.86; Chi ^m = 315.08, dir = 5 ($P < 0.00001$); P = 86%. Test for overall effect $Z = 8.16; (P = 315.08, dir = 5 (P < 0.00001); P = 86%.Test for overall effect Z = 9.16; (P = 315.08, dir = 5 (P < 0.00001); P = 82.%Fig. 5 Subgroup analysis of triple mixed infections by Plasmodium species between residents and febrile groups$	Perandin et al., 2004	1	62	1	62	1.5%	1.00 [0.06, 16.35]				
Rubio tal. (2), 19991898892.2%0.12 [0.01, 0.54]Provide tal. (2)145384743843.9%0.06 [0.02, 0.17]Provide tal. (2014)5384743843.9%0.06 [0.02, 0.14]Subtotal (95% C)3997399734.0%0.07 [0.04, 0.13]Total events961104Heterogeneity: Tau" = 0.56; Chi"= 37.40, dif = 9 ($\phi < 0.0001$); F= 78%Test for everal effect Z = 8.78 ($\phi < 0.0001$)1.3.2 ResidentsGabrielli et al., 201611641642.3%Marques et al., 2016116416584.7%Hopkins et al., 2016116581476584.6%Marques et al., 20069115801154.1%Mueller et al., 201611644.6%0.23 [0.16, 0.23]Hueler et al., 200691151564.5%0.13 [0.09, 0.36]Marques et al., 200691158444.5%0.13 [0.09, 0.36]Stall et al., 201574.744.24.744.1%0.05 [0.02, 0.13]Stall et al., 2019102.2904.0%0.30 [0.13, 0.72]Stall et al., 2019102.92.94.2%0.16 [0.08, 0.22]Stall et al., 2019102.9932.294.2%0.16 [0.08, 0.22]Stall et al., 2019102.92.94.2%0.16 [0.08, 0.22]Stall et al., 2019102.92.94.2% </td <td>Putaporntip et al., 2009</td> <td>11</td> <td>1751</td> <td>230</td> <td>1751</td> <td>4.4%</td> <td>0.04 [0.02, 0.08]</td> <td></td> <td></td> <td></td>	Putaporntip et al., 2009	11	1751	230	1751	4.4%	0.04 [0.02, 0.08]				
Rubic et al., 1999 3 126 41 126 3.4% 0.05 [0.02, 0.14] Stud et al., 2014 5 3997 3.997 3.997 3.0% 0.06 [0.02, 0.14] Stud et al., 2015 3997 3.997 3.0% 0.06 [0.02, 0.14] \bullet Heterogenely, Tau ⁺ = 0.56; Chi ⁺ = 37.40, df = 9 ($P < 0.0001$); P= 76% Test for overall effect. Z= 8.78 ($P < 0.00001$); P= 76% Tast events 6 1104 16 164 2.3% 0.06 [0.01, 0.43] Hopkins et al., 2016 1 164 16 164 2.3% 0.06 [0.01, 0.43] Hopkins et al., 2015 1 165 147 658 4.5% 0.23 [0.16, 0.33] + May et al., 1209 27 165 53 165 4.5% 0.41 [0.24, 0.70] + Mehotra et al., 2002 146 1844 4.5% 0.21 [0.01, 0.3, 0] + + May et al., 1939 27 165 53 165 4.5% 0.21 [0.01, 0.2, 0] + Pongroungs et al., 2014 5 368 76 168 0.15 [0.07, 0.36] + +	Rubio et al. (2), 1999	1	89	8	89	2.2%	0.12 [0.01, 0.94]				
Zhou et al., 2014 5 384 74 384 3.9% 0.06 [0.02, 0.14] Total events 95 1104 Heterogeneix, Tur* 0.56; Chi" = 37.40, df = 9 (P < 0.0001); P = 76%	Rubio et al., 1999	3	126	41	126	3.4%	0.05 [0.02, 0.17]				
Total events 96 1104 Heterogeneily: Tau" = 0.56; Ch" = 37.40, df = 9 (P < 0.0001); P = 76% Test for overall effect Z = 8.78 (P < 0.00001) 1.3.2 Residents Gabrielli et al., 2016 1 164 16 164 2.3% 0.06 [0.01, 0.43] Hopkins et al., 2003 1 165 147 658 4.6% 0.23 [0.16, 0.33] + Marques et al., 2005 9 115 80 115 4.1% 0.04 [0.02, 0.08] + Meltoris et al., 2002 33 541 182 541 4.5% 0.03 [0.1, 0.43] + Meltoris et al., 2009 146 1844 660 1844 4.8% 0.20 [0.16, 0.24] + Pongvongs et al., 2018 8 90 2.2 90 4.0% 0.03 [0.10, 0.2] + Steenkeste et al., 2010 10 102 27 102 4.1% 0.05 [0.02, 0.13] + Steenkeste et al., 2019 51 618 676 388 3.9% 0.05 [0.02, 0.13] + Steenkeste et al., 2019 51 618 618 4.7%	Zhou et al., 2014 Subtotal (95% CI)	5	384 3997	74	384 3997	3.9% 34.0 %	0.06 [0.02, 0.14] 0.07 [0.04, 0.13]	•			
Heterogeneity: Tau" = 0.56; Chi" = 37.40, df = 9 ($P < 0.0001$); P = 76% Test for overall effect: Z = 8.78 ($P < 0.0001$) 1.3.2 Residents Cabrielli et al., 2016 1 164 16 164 2.3% 0.06 [0.01, 0.43] Hopkins et al., 2016 1 1658 147 658 4.6% 0.23 [0.16, 0.33] Kasehagen et al., 2006 41 658 147 658 4.6% 0.24 [0.24, 0.70] Margues et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] Helholtra et al., 2015 7 474 42 474 4.1% 0.15 [0.07, 0.35] Hougings et al., 2016 7 4774 42 474 4.1% 0.15 [0.07, 0.35] Steenkeste et al., 2010 10 102 27 102 4.1% 0.30 [0.14, 0.66] Subissi et al., 2010 10 102 27 102 4.1% 0.30 [0.14, 0.66] Jong and 1.201 2 117 24 117 3.0% 0.07 [0.02, 0.29] Heterogeneity: Tau" = 0.42; Chi" = 110.18, df = 15 ($P < 0.0001$); $F = 86\%$ Test for overall effect: Z = 8.65 ($P < 0.00001$) For (195% CI) 10005 100.0% 0.14 [0.09, 0.21] For (195% CI) 10005 100.0% 0.14 [0.09, 0.21] Heterogeneity: Tau" = 0.96; Chi" = 315.09, df = 25 ($P < 0.00001$); $F = 88.2\%$ Fig. 5 Subgroup analysis of triple mixed infections by <i>Plasmodium</i> species between residents and febrile groups	Total events	96		1104							
Test for overall effect: Z = 8.78 (P < 0.00001)	Heterogeneity: Tau ² = 0.56; Cł	ni² = 37.40, d	f=9(P <	< 0.0001); I	² =76%						
1.3.2 Residents Gabrielli et al., 2016 1 164 16 164 2.3% 0.06 [0.01, 0.43] Hopkins et al., 2013 10 199 46 199 4.2% 0.18 [0.09, 0.36] Kasehagen et al., 2006 41 658 147 658 4.6% 0.23 [0.16, 0.33] Margues et al., 2005 9 115 80 115 4.1% 0.04 [0.24, 0.70] Mehotra et al., 2009 146 1844 560 1844 4.8% 0.20 [0.13, 0.24] + Pongyongse et al., 2018 8 90 22 90 4.0% 0.30 [0.13, 0.72] + Stall et al., 2016 7 474 4.2 474 4.1% 0.15 [0.07, 0.35] + Stall et al., 2016 7 474 4.2 474 4.1% 0.30 [0.14, 0.66] + Steenkeste et al., 2001 10 102 27 102 4.1% 0.30 [0.14, 0.66] + Toma et al., 2010 10 29 53 299 4.2% 0.16 [0.08, 0.21] + Total events 413	Test for overall effect: Z = 8.78	(P < 0.0000	1)								
Gabrielli et al., 2016 1 164 16 164 2.3% 0.06 [0.01, 0.43] Hopkins et al., 2013 10 199 46 199 4.2% 0.18 [0.09, 0.36] Margues et al., 2006 41 658 147 658 4.6% 0.23 [0.16, 0.3] Margues et al., 2006 9 115 80 115 4.1% 0.04 [0.02, 0.08] Margues et al., 2002 33 541 182 541 4.6% 0.13 [0.09, 0.19] Mehiotra et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] Pongyongsa et al., 2018 8 90 22 90 4.0% 0.30 [0.13, 0.72] Sitali et al., 2015 7 474 42 474 4.1% 0.15 [0.07, 0.35] Sitenkeste et al., 2010 10 102 27 102 4.1% 0.30 [0.14, 0.66] Torma et al., 2010 10 122 27 102 4.1% 0.30 [0.14, 0.66] Zhou et al., 1988 52 114 36 114 4.5% 0.22 [1.06, 3.12] Subitotal	1.3.2 Residents										
$\begin{array}{l lllllllllllllllllllllllllllllllllll$	Gabrielli et al., 2016	1	164	16	164	2.3%	0.06 [0.01, 0.43]				
Kasehagen et al., 2006 41 658 147 658 4.6% 0.23 [0.16, 0.33]	Hopkins et al., 2013	10	199	46	199	4.2%	0.18 (0.09, 0.36)	—			
$\begin{array}{l c c c c c c c c c c c c c c c c c c c$	Kasehagen et al., 2006	41	658	147	658	4.6%	0.23 [0.16, 0.33]	-			
May et al., 1999 27 165 53 165 4.5% 0.41 10.24, 0.70) Mehlotra et al., 2002 33 541 182 541 4.6% 0.13 [0.09, 0.19] Mueller et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] + Pongvongsa et al., 2018 8 90 22 90 4.0% 0.30 [0.13, 0.72] + Sitali et al., 2016 7 474 42 474 4.1% 0.15 [0.02, 0.13] + Steenkeste et al., 2019 31 140 71 140 4.5% 0.28 [0.16, 0.46] + Toma et al., 2001 2 117 24 117 0.0% 0.07 [0.02, 0.29] + Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] + Total events 443 1602 6008 66.0% 0.20 [0.14, 0.29] + Total events 539 2706 - - - - - <td>Marques et al., 2005</td> <td>9</td> <td>115</td> <td>80</td> <td>115</td> <td>4.1%</td> <td>0.04 [0.02, 0.08]</td> <td>—</td> <td></td> <td></td>	Marques et al., 2005	9	115	80	115	4.1%	0.04 [0.02, 0.08]	—			
$\begin{array}{l c c c c c c c c c c c c c c c c c c c$	May et al., 1999	27	165	53	165	4.5%	0.41 [0.24, 0.70]				
Mueller et al., 2009 146 1844 560 1844 4.8% 0.20 [0.16, 0.24] + Pongwongs et al., 2018 8 90 22 90 4.0% 0.30 [0.13, 0.72] + Stali et al., 2015 7 474 42 474 4.1% 0.15 [0.07, 0.35] + Steenkeste et al., 2009 31 140 71 140 4.5% 0.28 [0.16, 0.46] + Steenkeste et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] + Toma et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] + Zhou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] + Subtotal (95% Cl) 6008 6008 60.0% 0.20 [0.14, 0.29] + + Total events 443 1602 + + + + + + + + Total events 539 2706 + + + + + + + + + <td< td=""><td>Mehlotra et al., 2002</td><td>33</td><td>541</td><td>182</td><td>541</td><td>4.6%</td><td>0.13 (0.09, 0.19)</td><td>-</td><td></td><td></td></td<>	Mehlotra et al., 2002	33	541	182	541	4.6%	0.13 (0.09, 0.19)	-			
Pongvongs at et al., 2018 8 90 22 90 4.0% 0.30 [0.13, 0.72] Sitali et al., 2015 7 474 42 474 4.1% 0.15 [0.07, 0.35] Slugdts et al., 2014 5 368 76 368 76 308 0.05 [0.02, 0.13] Steenkeste et al., 2009 31 140 71 140 4.5% 0.28 [0.16, 0.46] Steenkeste et al., 2010 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Subtotal (95% CI) 6008 6008 6008 6008 0.20 [0.14, 0.29] 4.2% Total events 443 1602 1.82 [1.06, 3.12] 4.3% 4.43 1602 Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 92% 0.14 [0.09, 0.21] 4.3% 6.01 0.11	Mueller et al., 2009	146	1844	560	1844	4.8%	0.20 [0.16, 0.24]	+			
Sitali et al., 2015 7 474 42 474 4.1% 0.15 [0.07, 0.35] Sluydts et al., 2014 5 368 76 368 3.9% 0.05 [0.02, 0.13] Steenkeste et al., 2009 31 140 71 140 4.5% 0.28 [0.16, 0.46] Steenkeste et al., 2010 10 102 27 102 4.1% 0.30 [0.14, 0.66] Subissi et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Tom at al., 2011 2 117 24 117 3.0% 0.07 [0.02, 0.29] Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Stubtotal (95% CI) 6008 6008 66.0% 0.20 [0.14, 0.29] + Test for overall effect: Z = 8.65 (P < 0.00001)	Pongvongsa et al., 2018	8	90	22	90	4.0%	0.30 [0.13, 0.72]				
Sluydts et al., 2014 5 368 76 368 3.9% 0.05 [0.02, 0.13] Steenkeste et al., 2009 31 140 71 140 4.5% 0.28 [0.16, 0.46] Steenkeste et al., 2010 10 102 2.7 102 4.1% 0.30 [0.14, 0.66] Subissi et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Substotal (95% Cl) 6008 6008 66.0% 0.20 [0.14, 0.29] + Total events 443 1602 + + + Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% 0.14 [0.09, 0.21] + Total (95% Cl) 10005 10005 0.04 0.14 [0.09, 0.21] + Total (95% Cl) 10005 10005 0.0001); I ² = 92% - - - Test for overall effect: Z = 9.16 (P < 0.00001)	Sitali et al., 2015	7	474	42	474	4.1%	0.15 [0.07, 0.35]				
Steenkeste et al., 2009 31 140 71 140 4.5% 0.28 [0.16, 0.46] Steenkeste et al., 2010 10 102 27 102 4.1% 0.30 [0.14, 0.66] Subissi et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2010 2 117 24 117 3.0% 0.07 [0.02, 0.29] Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Zhou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] Subtotal (95% CI) 6008 6008 66.0% 0.20 [0.14, 0.29] - Total events 443 1602 Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); i ² = 86% - - - Total (95% CI) 10005 100.0% 0.14 [0.09, 0.21] - - Total (95% CI) 10005 100.0% 0.14 [0.09, 0.21] - - - Total events 539 2706 - - - - - - <td< td=""><td>Sluydts et al., 2014</td><td>5</td><td>368</td><td>76</td><td>368</td><td>3.9%</td><td>0.05 [0.02, 0.13]</td><td></td><td></td><td></td></td<>	Sluydts et al., 2014	5	368	76	368	3.9%	0.05 [0.02, 0.13]				
Steenkeste et al., 2010 10 102 27 102 4.1% 0.30 [0.14, 0.66] Subissi et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2001 2 117 24 117 3.0% 0.07 [0.02, 0.29] Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Zhou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] Subtotal (95% CI) 6008 6008 66.0% 0.20 [0.14, 0.29] • Total events 443 1602 Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% • • Total (95% CI) 10005 10005 0.14 [0.09, 0.21] • Total events 539 2706 • • • Heterogeneity: Tau ² = 0.96; Chi ² = 315.09, df = 25 (P < 0.00001); I ² = 92% • • • Test for overall effect: Z = 9.16 (P < 0.00001)	Steenkeste et al., 2009	31	140	71	140	4.5%	0.28 (0.16, 0.46)				
Subissi et al., 2019 10 299 53 299 4.2% 0.16 [0.08, 0.32] Toma et al., 2001 2 117 24 117 3.0% 0.07 [0.02, 0.29] Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Zhou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] Subtotal (95% Cl) 6008 6008 66.0% 0.20 [0.14, 0.29] + Test for overall effect: Z = 8.65 (P < 0.00001); I ^a = 86% Test for overall effect: Z = 8.65 (P < 0.00001); I ^a = 92% + + Test for overall effect: Z = 9.16 (P < 0.00001)	Steenkeste et al., 2010	10	102	27	102	4.1%	0.30 [0.14, 0.66]				
Toma et al., 2001 2 117 24 117 3.0% 0.07 [0.02, 0.29] Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Shou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] Subtotal (95% CI) 6008 6008 66.0% 0.20 [0.14, 0.29] - Total events 443 1602 - - - Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% - - - Test for overall effect: Z = 8.65 (P < 0.00001)	Subissi et al., 2019	10	299	53	299	4.2%	0.16 [0.08, 0.32]				
Woldearegai et al., 2019 51 618 167 618 4.7% 0.24 [0.17, 0.34] Zhou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] Subtotal (95% CI) 6008 6008 60.0% 0.20 [0.14, 0.29] Total events 443 1602 Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% Test for overall effect: Z = 8.65 (P < 0.00001)	Toma et al., 2001	2	117	24	117	3.0%	0.07 [0.02, 0.29]				
Zhou et al., 1998 52 114 36 114 4.5% 1.82 [1.06, 3.12] Subtotal (95% CI) 6008 6008 66.0% 0.20 [0.14, 0.29] Total events 443 1602 Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% Test for overall effect: Z = 8.65 (P < 0.00001) 0005 10005 0.04 0.14 [0.09, 0.21] Total events 539 2706 Heterogeneity: Tau ² = 0.96; Chi ² = 315.09, df = 25 (P < 0.00001); I ² = 92% 0.01 0.1 10 100 Test for overall effect: Z = 9.16 (P < 0.00001) Favours [experimental] Favours [control] Fig. 5 Subgroup analysis of triple mixed infections by <i>Plasmodium</i> species between residents and febrile groups	Woldearegai et al., 2019	51	618	167	618	4.7%	0.24 [0.17, 0.34]	-			
Total events 443 1602 Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% Test for overall effect: Z = 8.65 (P < 0.00001)	Zhou et al., 1998 Subtotal (95% Cl)	52	114 6008	36	114 6008	4.5% 66.0%	1.82 [1.06, 3.12] 0.20 [0.14, 0.29]	◆ 「			
Heterogeneity: Tau ² = 0.42; Chi ² = 110.18, df = 15 (P < 0.00001); I ² = 86% Test for overall effect: Z = 8.65 (P < 0.00001)	Total events	443		1602							
Total (95% Cl)1000510005100.0%0.14 [0.09, 0.21]Total events5392706Heterogeneity: Tau ² = 0.96; Chi ² = 315.09, df = 25 (P < 0.00001); l ² = 92% 0.01 0.1 100 Test for overall effect: Z = 9.16 (P < 0.00001)	Heterogeneity: Tau² = 0.42; Chi² = 110.18, df = 15 (P < 0.00001); l² = 86% Test for overall effect: Z = 8.65 (P < 0.00001)										
Total events5392706Heterogeneity: Tau ² = 0.96; Chi ² = 315.09, df = 25 (P < 0.00001); l ² = 92% 0.01 1 Test for overall effect: Z = 9.16 (P < 0.00001)	Total (95% CI)		10005		10005	100.0%	0.14 [0.09, 0.21]	•			
Heterogeneity: Tau ² = 0.96; Chi ² = 315.09, df = 25 (P < 0.00001); l ² = 92% 1 <td< td=""><td>Total events</td><td>539</td><td></td><td>2706</td><td></td><td></td><td>-</td><td>-</td><td></td><td></td></td<>	Total events	539		2706			-	-			
Test for overall effect: Z = 9.16 (P < 0.00001)	Heterogeneity: Tau ² = 0.96: Chi ² = 315.09. df = 25 (P < 0.00001); i ² = 92%										
Fig. 5 Subgroup analysis of triple mixed infections by <i>Plasmodium</i> species between residents and febrile groups	Test for overall effect: Z = 9.16 Test for subgroup differences	(P < 0.0000 Chi ² = 8.47.	1) df = 1 (F	P = 0.004).	l ² = 88.29	%	F	0.01 0.1 1 avours (experimental)	10 1 Favours (control)	00	
	Fig. 5 Subgroup analysis of	of triple mix	ked infe	ections by	/ Plasm	odium s	pecies between resi	dents and febrile gro	oups		

number of articles have reported on age and susceptibility to triple mixed infection. The included study by Camargo-Ayala et al. showed that patients tend to have a risk of triple mixed infection at an age range of 18–60 years than at \leq 5 or 5–18 years, whereas patients tend to have a risk of double infection at age range greater than 60 years than at \leq 5, 5–18 and 18–60 years [5]. However, small sample sizes of the triple and dual mixed infection were calculated for the risk estimate in the same study. Therefore, the association between age and different types of mixed infection (double and triple infection) should be analysed in further observational studies using the research gap in age and type of mixed infection.

The study demonstrated that vomiting and the intense brown colour of urine were associated with triple mixed infection. Regarding the geographical region analysed, triple mixed infection was mostly found at the Loretoyacu River in the Colombian Amazon region [5]. The high prevalence of triple mixed infection at the Loretoyacu River may be due to the occurrence of

the mosquito Anopheles maculatus, which can serve as a single vector for *P. falciparum/P. vivax/P. malariae* [5]. Triple species infection of *P. falciparum* and *P. malariae*, followed by *P. ovale* delayed infection, were also observed in two adopted children from the Central African Republic and may be attributed to late therapeutic failure or the relatively insufficient dosage due to increased oral clearance of atovaquone in paediatric patients [37].

The subgroup analysis demonstrated that the proportion of triple mixed infection was the highest in Oceania (23%) and Europe (21%) but the lowest in America. A previous study indicated that, in Oceania, where intense transmission occurs in very small focal forests or forest fringe areas, mixed infection are common but require submicroscopic detection [9]. Malaria disease in Europe has been mostly eradicated, but the increase in the number of imported malaria due to tourism, as well as population migration, resulted in increased mortality, from 3.8 to 20% [38]. These imported cases have increased the

	Triple infe	ctions	Dual infe	tions		Odds Ratio	Odds Ratio	Risk of Bias
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl	ABCDEFG
1.4.1 EDTA blood								
Calderaro et al., 2008	2	159	4	159	2.1%	0.49 [0.09, 2.73]		
Camargo-Ayala et al., 2016	14	531	225	531	3.8%	0.04 [0.02, 0.06]	- -	
Dhangadamajhi et al., 2009	5	197	79	197	3.3%	0.04 [0.02, 0.10]	<u> </u>	
Dormond et al., 2010	1	89	5	89	1.7%	0.19 [0.02, 1.67]		
Hopkins et al., 2013	10	199	46	199	3.6%	0.18 [0.09, 0.36]	_ -	
Kasehagen et al., 2006	41	658	147	658	4.1%	0.23 [0.16, 0.33]		
Lorenzetti et al., 2008	1	115	30	115	1.8%	0.02 [0.00, 0.19]	←	
Marques et al., 2005	9	115	80	115	3.5%	0.04 [0.02, 0.08]	<u> </u>	
May et al., 1999	27	165	53	165	3.9%	0.41 [0.24, 0.70]	<u> </u>	
Mehlotra et al., 2000	41	163	63	163	4.0%	0.53 [0.33, 0.86]		
Mehlotra et al., 2002	33	541	182	541	4.0%	0.13 [0.09, 0.19]	—	
Mueller et al., 2009	146	1844	560	1844	4.2%	0.20 [0.16, 0.24]	+	
Niño et al., 2016	52	596	480	596	4.1%	0.02 [0.02, 0.03]		
Perandin et al., 2004	1	62	1	62	1.2%	1.00 [0.06, 16.35]		
Rubio et al., 1999	1	89	8	89	1.7%	0.12 (0.01, 0.94)		
Toma et al., 2001	2	117	24	117	2.5%	0.07 [0.02, 0.29]		
Woldearegai et al., 2019	51	618	167	618	4.1%	0.24 [0.17, 0.34]		
Subtotal (95% CI)		6258		6258	53.6%	0.13 [0.08, 0.21]	◆	
Total events	437		2154					
Heterogeneity: Tau ² = 0.89; Ch	ni ² = 227.42.	df = 16 (P < 0.0000	1); I ² = 9;	3%			
Test for overall effect: Z = 7.85	(P < 0.0000	1)						
1.4.2 Dried blood spot								
Asua et al., 2017	1	474	37	474	1.8%	0.02 [0.00, 0.18]	←────	
Fuehrer et al., 2010	2	189	23	189	2.5%	0.08 [0.02, 0.33]		
Gabrielli et al., 2016	1	164	16	164	1.8%	0.06 [0.01, 0.43]	←	
Jiang et al., 2010	2	146	36	146	2.5%	0.04 [0.01, 0.18]		
Krishna et al., 2017	6	353	62	353	3.4%	0.08 [0.03, 0.19]		
Pati et al., 2017	5	110	18	110	3.1%	0.24 [0.09, 0.68]		
Pongvongsa et al., 2018	8	90	22	90	3.4%	0.30 [0.13, 0.72]		
Putaporntip et al., 2009	11	1751	230	1751	3.8%	0.04 [0.02, 0.08]	<u> </u>	
Rubio et al. (2), 1999	3	126	41	126	2.9%	0.05 [0.02, 0.17]		
Sitali et al., 2015	7	474	42	474	3.5%	0.15 (0.07, 0.35)		
Sluydts et al., 2014	5	368	76	368	3.3%	0.05 [0.02, 0.13]		
Steenkeste et al., 2009	31	140	71	140	3.9%	0.28 [0.16, 0.46]	- -	
Steenkeste et al., 2010	10	102	27	102	3.5%	0.30 [0.14, 0.66]		
Subissi et al., 2019	10	299	53	299	3.7%	0.16 [0.08, 0.32]	<u> </u>	
Zhou et al., 2014	5	384	74	384	3.3%	0.06 [0.02, 0.14]		
Subtotal (95% CI)		5170		5170	46.4%	0.10 [0.07, 0.17]	◆	
Total events	107		828					
Heterogeneity: Tau ² = 0.55; Ch	ni² = 51.81, d	lf = 14 (P	< 0.00001); l² = 73'	%			
Test for overall effect: Z = 9.58	(P < 0.0000	1)						
Total (95% CI)		11428		11428	100.0%	0.11 [0.08, 0.16]	◆	
Total events	544		2982					
Heterogeneity: Tau ² = 0.78; Ch	n [*] = 281.40,	df = 31 (P < 0.0000	1); l² = 8!	9%		0.01 0.1 1 10	100
Test for overall effect: Z = 11.8	9 (P < 0.000	01)				F	avours (experimental) Favours (con	trol]
lest for subgroup differences:	Chi [*] = 0.29	, αf = 1 (F	' = 0.59), l ²	= 0%		1 1 A		
Fig. 6 Subgroup analysis o	ot triple mi	xed infe	ections by	' Plasm	odium s	pecies between the	two blood collection methods	

number of malaria cases in places where its transmission was low or previously eradicated, such as in Europe [39].

Knowledge about mixed infection is important not only to develop appropriate control measures but also for therapeutic options. For example, if *P. vivax* infection is suppressed by mixed infection with *P. falciparum*, effective control of *P. falciparum* infection in an area will activate *P. vivax* transmission in the community, a condition that is more difficult to control [17]. The present study was limited by the heterogeneity of the included studies and should be interpreted cautiously. Thus, the findings of the present study might not necessarily apply to all co-endemic regions. The present study could not extract the age of patients with triple mixed infection due to the lack of data reported in the included studies. Moreover, the clinical data, laboratory data, and treatment data of individual patients with triple mixed infection were also unavailable to extract. These data should be included and declared in malaria studies for its apparent value in cases of review and meta-analyses. Future meta-analyses should assess the cases reported or case series to provide a greater understanding of the factors associated with triple mixed infection.

Conclusion

In summary, although mixed infection was recognized, the prevalence of triple mixed infection was high (4%). The proportion of triple mixed infection was the highest in Oceania and Europe but lower in America. Compared with the proportion of double mixed infection, triple

	Triple infections Dual infections		Odds Ratio		Odds Ratio	Risk of Bias				
Study or Subgroup	Events	Total	Events	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl	ABCDEFG		
1.7.1 ≤5 years										
Barber et al., 2012	1	445	1	445	4.5%	1.00 [0.06, 16.04]				
Camargo-Ayala et al., 2016	4	531	16	531	9.6%	0.24 [0.08, 0.74]				
Dhangadamajhi et al., 2009	1	197	7	197	6.1%	0.14 [0.02, 1.14]				
Mehlotra et al., 2000	2	163	14	163	8.1%	0.13 [0.03, 0.59]				
Mehlotra et al., 2002	0	541	0	541		Not estimable				
Pongvongsa et al., 2018	4	90	3	90	8.0%	1.35 [0.29, 6.21]	-			
Subissi et al., 2019	5	299	27	299	10.1%	0.17 [0.07, 0.45]				
Subtotal (95% CI)		2266		2266	46.4%	0.27 [0.13, 0.56]	◆			
Total events	17		68							
Heterogeneity: Tau ² = 0.24; Ch	ni² = 7.22, df =	= 5 (P = I	0.20); l² = 3	1%						
Test for overall effect: Z = 3.55	(P = 0.0004)									
1.7.2 >5 years										
Barber et al., 2012	3	445	33	445	9.2%	0.08 (0.03, 0.28)				
Camargo-Ayala et al., 2016	10	531	210	531	11.1%	0.03 [0.02, 0.06]				
Dhangadamajhi et al., 2009	4	197	71	197	9.8%	0.04 [0.01, 0.10]	_ -			
Mehlotra et al., 2000	32	163	166	163		Not estimable				
Mehlotra et al., 2002	1	541	15	541	6.4%	0.06 [0.01, 0.49]				
Pongvongsa et al., 2018	3	90	2	90	7.0%	1.52 [0.25, 9.30]	•			
Subissi et al., 2019	5	299	26	299	10.0%	0.18 [0.07, 0.47]	_			
Subtotal (95% CI)		2266		2266	53.6%	0.09 [0.04, 0.25]	•			
Total events	58		523							
Heterogeneity: Tau ² = 1.12; Ch	ni² = 22.85, di	f= 5 (P =	= 0.0004); i ^s	²= 78%						
Test for overall effect: Z = 4.66	(P < 0.00001	1)								
Total (95% CI)		4532		4532	100.0%	0.16 [0.08, 0.34]	◆			
Total events	75		591							
Heterogeneity: Tau ² = 1.18; Chi ² = 45.26, df = 11 (P < 0.00001); l ² = 76%										
Test for overall effect: Z = 4.85 (P < 0.00001)										
Test for subgroup differences:	Chi ² = 2.83,	df = 1 (F	P = 0.09), I²	= 64.7%			avours (experimental) - Favours (con	aoj		
Fig. 7 Subgroup analysis o	f triple mix	ed infe	ctions by	Plasmo	<i>dium</i> sp	pecies between th	e two age groups			



mixed infection was higher in residents (20%) than in febrile patients (7%). The findings suggested that in some regions, co-endemic for triple mixed infection, PCR, or molecular diagnosis for all residents in communities where malaria is endemic can provide prevalence data and intervention measures, as well as prevent disease transmissions and enhance malaria elimination efforts.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12936-020-03292-8.

Additional file 1. Table S1.

Abbreviations

CI: Confidence interval; DNA: Deoxyribonucleic acid; NOS: Newcastle–Ottawa Scale; OR: Odds ratio; PCR: Polymerase chain reaction; PRISMA: Preferred reporting items for systematic reviews and meta-analyses; RDT: Rapid diagnostic test; WHO: World Health Organization.

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Authors' contributions

MK and KUK participated in the study design, data analysis, and writing of the paper. GDM and FRM participated in the writing of the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used during the current study are available without restriction.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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