Global status of *Toxoplasma gondii* infection and associated risk factors in people living with HIV: A systematic review and meta-analysis

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Abstract

Objective: *Toxoplasma* infection remains as the most common cause of focal brain lesions among people living with HIV (PLHIV) despite the decline in opportunistic infections with the introduction of antiretroviral treatment. This study was conducted to provide a summary of evidence about the seroprevalence of *Toxoplasma gondii* and prevalence of active *T. gondii* infection and associated risk factors among PLHIV.

Design: PRISMA guidelines were followed. Scopus, PubMed, Science Direct, and EMBASE were searched from 1997 to July 2018. All peer-reviewed original research articles describing *T. gondii* infection among PLHIV with different diagnostic methods were included.

Methods: Incoherence and heterogeneity between studies were quantified by *I2* index and Cochran's Q test. Publication and population bias were assessed with funnel plots and Egger's regression asymmetry test. All statistical analyses were performed using StatsDirect.

Results: A total of 111 studies from 37 countries assessing 66,139 blood samples were included in this study. The pooled prevalence of *T. gondii* infection among PLHIV was 3.24% by IgM and 26.22% by molecular methods using the random-effects model. Pooled seroprevalence of *T. gondii* by IgG was 44.22%. There was a relationship between *Toxoplasma* prevalence and gender, raw meat consumption, contact with cat and knowledge about toxoplasmosis.

Conclusion: High *Toxoplasma* seroprevalence among PLHIV observed in this study emphasizes the need for implementing screening and prophylaxis tailored to the local context. Owing to the serious and significant clinical manifestations of the parasite in case of reactivation, early identification of seropositivity for initiating prophylaxis among those with a CD4 count of <200cells/mL is recommended.

Key words: Toxoplasmosis, HIV, AIDS, Immunocompromised, Prevalence, *Toxoplasma gondii*

Introduction

The obligate intracellular protozoan *Toxoplasma gondii* (*T. gondii*), the causative agent of toxoplasmosis, is estimated to infect a third of the world's population [1]. It is also one of the commonest parasites infecting a wide variety of vertebrate hosts [2, 3]. Humans acquire the infection via ingestion of the tissue cysts in undercooked meat, oocysts in contaminated water or food, and congenitally [4, 5].

Primary *T. gondii* infection in immunocompetent patients largely remains asymptomatic. In the immunocompromised host, this opportunistic pathogen carries a potential risk of severe disease, especially among people living with HIV (PLHIV) and those with malignancy [6, 7]. PLHIV are not only at risk of reactivation but also at risk of severe disease following primary toxoplasmosis [8]. *Toxoplasma* has been reported as the most common cause of focal brain lesions among PLHIV [9]. Reactivation remains the predominant route by which *Toxoplasma* infection manifests especially among those with a CD4 cell count of below 200 cells/μL, and it carries a risk of fatal outcome if untreated [8, 10, 11].

The diagnosis of toxoplasmosis is based on clinical and radiological findings supported by serological tests such as enzyme-linked immunosorbent assay (ELISA) and immunofluorescence antibody assay (IFA) and/or detection of DNA by molecular techniques [1]. While toxoplasmosis IgG antibodies are a marker of chronic exposure, IgM antibodies and the presence of DNA suggests recent infection or reactivation and provides better estimates of active *T. gondii* infection [1, 12]. A systematic review previously assessed the worldwide seroprevalence of *T. gondii* among PLHIV based on *T. gondii* antibody [11].

By conducting a comprehensive search from 1997 to July 2018 we identified 37 additional studies, and by reporting IgM and molecular data, we provide summary estimates

of seroprevalence as well as the prevalence of active *T. gondii* infection and associated risk factors in PLHIV in this study.

Methods

A protocol for the review was devised following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13]. Our systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42019120598).

Search strategy

We retrieved all articles on *Toxoplasma* infection in PLHIV through systematic searches of major databases such as PubMed, Ovid, Web of Science, Scopus, Google Scholar and Cochrane from 1997 to 2018 using Medical Subject Headings (MeSH) terms including "*Toxoplasma gondii*" or "toxoplasmosis" and "seroprevalence", "serology" and "molecular" and "diagnosis" and "IgG" and "IgM" "immunocompromised" or "immunosuppressed" or "immunodeficiency" or "immune deficiency" or "HIV" or "AIDS" or "acquired immune deficiency syndrome". (Fig. 1).

Selection criteria

Articles were screened and selected for full-text review if they met the following selection criteria: (1) full text cross-sectional, case-control, and cohort studies that reported *T. gondii* among PLHIV; (2) studies presenting final results with raw data; (3) published papers in English; (4) published online from 1997 to 2018. Studies that did not include raw data, and where data from each participant was not independently retrievable was excluded. We also excluded studies if they were reviews, case reports, animal studies or duplicates or if the sample size was less than 20. Additionally, only one report was included if more than one

report was published from the same study. In order to provide recent and representative estimates, studies were excluded if they presented data collected prior to 1997. To identify additional published articles, we used the PubMed "related articles" option and checked the reference lists of the original and review articles

Data abstraction and tabulation

Two authors (HS and MZ) carefully screened references and retrieved articles according to the eligibility criteria. Any study that did not match the eligibility criteria was excluded. Two reviewers independently assessed the quality (JBI Critical Appraisal Checklist) and performed the final article selection[14]. Any disagreements with the selected studies were resolved by discussion and the involvement of an additional two authors. From each study, the following variables were extracted: the first author, the year of publication, location of study, duration of the study, sample size, diagnostic methods used, demographic characteristics, number of positive samples, and CD4 cell count in cells/μL. To gather the raw data, some authors had to be contacted.

Meta-analysis

For every study included, the point estimate and 95% confidence interval (CI) were calculated. A forest plot was generated to display the summarized results and heterogeneity among the included studies. The heterogeneity was expected in advance, and statistical analyses, including I2 and Cochrane's Q test were used to quantify variations. The random effects model [15] was performed for the meta-analysis using Stats Direct statistical software (http://www.statsdirect.com). The size of every square indicated the weight of every study and the crossed lines illustrated CI. A prediction interval was calculated using R software [16, 17].

Additional meta-analysis was performed to evaluate the risk factors for *T. gondii* infection: gender, level of education, knowledge about toxoplasmosis, residence, CD4 count (<200 cells/μL), contact with cats, raw meat or raw vegetable consumption and antiretroviral therapy. Heterogeneity in all meta-analyses was assessed using the I2 index and Cochran's Q test. All statistical analyses were performed using StatsDirect (Version 2.7.2).

Results

The systematic search identified 8,851 potentially relevant articles. After reviewing the eligibility criteria, a total of 111 studies from 37 countries assessing 66,139 blood samples were included. The number of selected papers at each step of the screening and eligibility is reported in the flow diagram (Fig. 1).

Baseline characteristics of the included studies using serological and molecular assays are shown in the supplementary material (Supplementary Table 1 and 2). Three types of diagnostic methods were used to detect the prevalence of *T. gondii* in included studies: IgM (n=41), IgG (n=97) and molecular methods (n=19).

The pooled prevalence of *T. gondii* infection among PLHIV was 3.24% (95% CI =1.69-5.28%) (Q=1004.004653, df=40, I2 =96%, *P*<0.0001) by IgM (and 26.22% (95%CI=15.57-38.51%) (Q=718.624081, df=18, I2=97.5%, *P*<0.0001) by molecular methods. Pooled seroprevalence of *T. gondii* detected by IgG was 44.22% (95% CI=37.99-50.52%) (Q=11871.31, df=96, I2=99.2%, *P*<0.0001) however, there was a wide variation in the seroprevalence estimation among different studies (Supplementary figures). The prediction intervals for IgG, IgM, and PCR were 42.68 [7.16-87.79], 2.74 [0. 23-2.59], and 25.99 [5.64-67. 36], respectively. The pooled prevalence of *Toxoplasma* among PLHIV in different countries is demonstrated in Fig. 2.

Of the 111 studies, 27 studies have reported gender, 11 reported raw meat consumption, 11 reported contact with a cat, ten reported the level of education and ten reported antiretroviral therapy. A positive association was observed between *Toxoplasma* prevalence among PLHIV and female gender, raw meat consumption, contact with cat and knowledge about Toxoplasmosis ($p<0.005$) (Supplementary Table 3).

Discussion

This systematic review and meta-analysis provide comprehensive data on the seroprevalence of *T. gondii* and the prevalence of active infection among PLHIV. Our findings highlight the high global burden of *T. gondii* in PLHIV. The median worldwide seroprevalence of *T. gondii* was 44.22%, and the prevalence of active *T. gondii* infection was 3.24% by IgM and 26.22% by molecular methods.

Higher susceptibility to *Toxoplasma* infection PLHIV in this population [18, 19] has been ascribed to impaired IL-12 and IFN gamma production and cytotoxic T-lymphocyte activity irrespective of CD4 cell count [8, 20, 21]. Therefore, appropriate prevention, diagnosis and management of *T. gondii* infection among PLHIV require clinicians to be informed about the seroprevalence and distribution of active toxoplasmosis in a given setting [19]. In this study, we demonstrated that approximately half of the PLHIV were seropositive for *T. gondii*. After the primary infection, anti-*Toxoplasma* IgG antibodies start increasing and gradually decline over 1-2 years but can persist for life [8, 22, 23]. It is well known that IgG seropositive patients are at a higher risk of developing cerebral toxoplasmosis when CD4 counts fall below 200 cells/μL, and co-infection enhances immune impairment contributing to the clinical progression of HIV [24]. These findings highlight that *T. gondii* remains a significant risk for opportunistic infections among PLHIV, especially in sub-Saharan Africa, where access to HIV treatment remains sub-optimal.

According to this study, seropositivity varies world-wide depending on gender, living in urban areas, proximity to cats and consumption of raw meat, which is similar to the pattern observed in the immunocompetent population[25]. In a US based study, in asymptomatic healthy adults and children (aged 6-49 years old) and women (15-44 years old), the seroprevalence of *T. gondii* was 10.8% and 11.0%, respectively, which showed downtrend in the past decade[26]. Although based on limited data, *T. gondii* seroprevalence among asymptomatic healthy adults in the African continent appears to be high[25], suggesting that the seroprevalence rates may not significantly vary between PLHIV and non-HIV population in developing countries.

Our meta-analysis showed that only 3.24% of PLHIV had reactive anti-*Toxoplasma* IgM. IgM indicates recently acquired infection or reactivation, and it reaches a peak level at 1–2 months after infection and diminishes after 8 months in immunocompetent individuals [27]. In immunocompromised patients, it has a short half-life making IgM an unreliable marker of recent or active infection [1, 28]. *T. gondii* PCR in peripheral blood yields good sensitivity (up to 95.5%) and may enable rapid identification of *T. gondii* for early non-invasive identification of cerebral toxoplasmosis [29]. Several studies have demonstrated the applicability of plasma PCR for the diagnosis of cerebral toxoplasmosis [30]. PCR testing in CSF, on the other hand, has been shown to have lower sensitivity (<50%) [31]. In this systematic review, among 19 studies that reported molecular findings, one-third of PLHIV had the presence of DNA in plasma. While this reported prevalence of active infection is high, the numbers are low, and the majority of studies assessing molecular findings included patients with suspected cerebral toxoplasmosis.

This meta-analysis has several strengths. This is the most comprehensive study reporting differences in *T. gondii* prevalence in PLHIV according to the different diagnostic methods used. However, the true prevalence may be lower than observed in this study, as we

included “toxoplasmosis” as one of the keywords in our search. Our meta-analysis was limited by the potential effect of publication bias. The random effect model incorporates some of the heterogeneity, and high heterogeneity is not uncommon in meta-analyses of prevalence [32]. Despite the limitations, this systematic review provides the first summary estimates of IgM and molecular data in PLHIV and emphasizes that IgM should be used in caution in this population.

In conclusion, the high prevalence of *Toxoplasma* IgG seropositivity observed in this study emphasises the need for implementing screening and prophylaxis tailored to the local context for PLHIV. Seronegative patients should be educated to perform hand hygiene and avoid contact with raw or undercooked meat, drinking untreated water and handling cat litter boxes. Seropositive patients with a CD4 count below 200 should receive prophylaxis. IgM has a little value in this population as a marker of acute infection. Further work is required to define the role of plasma PCR for the diagnosis of cerebral toxoplasmosis.

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Conflict of interest

Authors declare that there is no conflict of interest.

Contribution statement

EA, HS, AB and MZ conceptualised this paper, HS, KH, ME and ASP independently assessed the quality and performed the final article selection, EA, MTR, and TJK performed the statistical analysis. EA, HB, FS drafted the first and MC drafted subsequent versions of the manuscript, HS, MC and MZ contributed equally to this manuscript, and all authors contributed to the final manuscript.

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Figure Legends:

Figure 1. Flowchart describing the study design process

Figure 2. Overall *Toxoplasma* prevalence among PLHIV in different geographical regions.

Supplementary figures:

Figure 1: Forest plot diagram of the studies reporting *Toxoplasma* IgG antibody among PLHIV

Figure 2. Forest plot diagram of the studies reporting *Toxoplasma* IgM antibody among PLHIV

Figure 3. Forest plot diagram of the studies reporting *Toxoplasma* infection based on molecular methods among PLHIV

Table . Subgroup analysis of the prevalence of *Toxoplasma* infection in HIV patients in different continents

Continent	No. of studies	Type of study	Prevalence (95% CI)	I_2	Heterogeneity		Egger test	
					Q	*P* value	T	*P* value
Africa	23	Serology (IgG)	60.18 (46.14 to 73.42)	98.8%	1893.5	< 0.0001	-3.15	0.6599
	14	Serology (IgM)	1.97 (0.72 – 3.8)	86.7%	97.83	< 0.0001	2.22	0.0039
	3	PCR	19.21 (0.22 – 66.58)	99.3%	287.74	< 0.0001	-	-
America	17	Serology (IgG)	42.16 (28.2–56.78)	99.4%	2850.7	< 0.0001	17.18	0.0017
	5	Serology (IgM)	0.27 (0.01 – 0.83)	0%	2.87	0.579	0.28	0.3758
	6	PCR	38.41 (28.94 – 48.34)	84.6%	32.43	< 0.0001	5.43	0.1291
Asia	43	Serology (IgG)	34.51 (27.18 –42.23)	98.6%	3085.3	< 0.0001	11.77	< 0.0001
	20	Serology (IgM)	5.76 (2.6– 10.05)	96.8%	591.69	< 0.0001	4.38	0.0009
	5	PCR	20.03 (8.04 – 35.72)	91.2%	45.62	< 0.0001	-1.98	0.7608
Europe	13	Serology (IgG)	50.07 (33.99 – 66.14)	99.2%	1571.1	< 0.0001	16	0.0032
	2	Serology (IgM)	0.48 (0.2–3.38)	-	25.34	< 0.0001	-	-
	5	PCR	22.76 (2.89– 53.81)	97.9%	192.05	< 0.0001	7.27	0.146

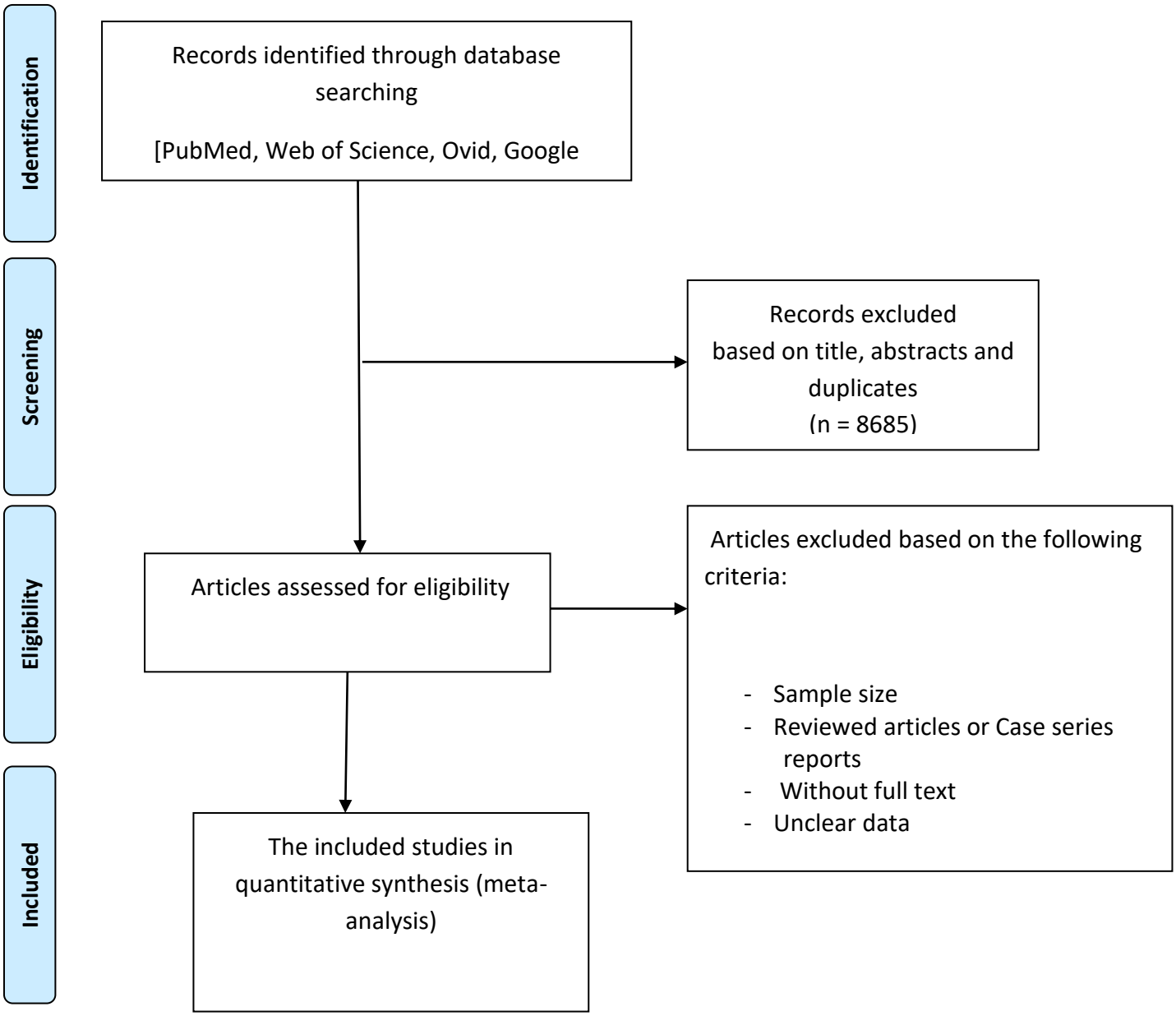


Figure 1. Flowchart describing the study design process

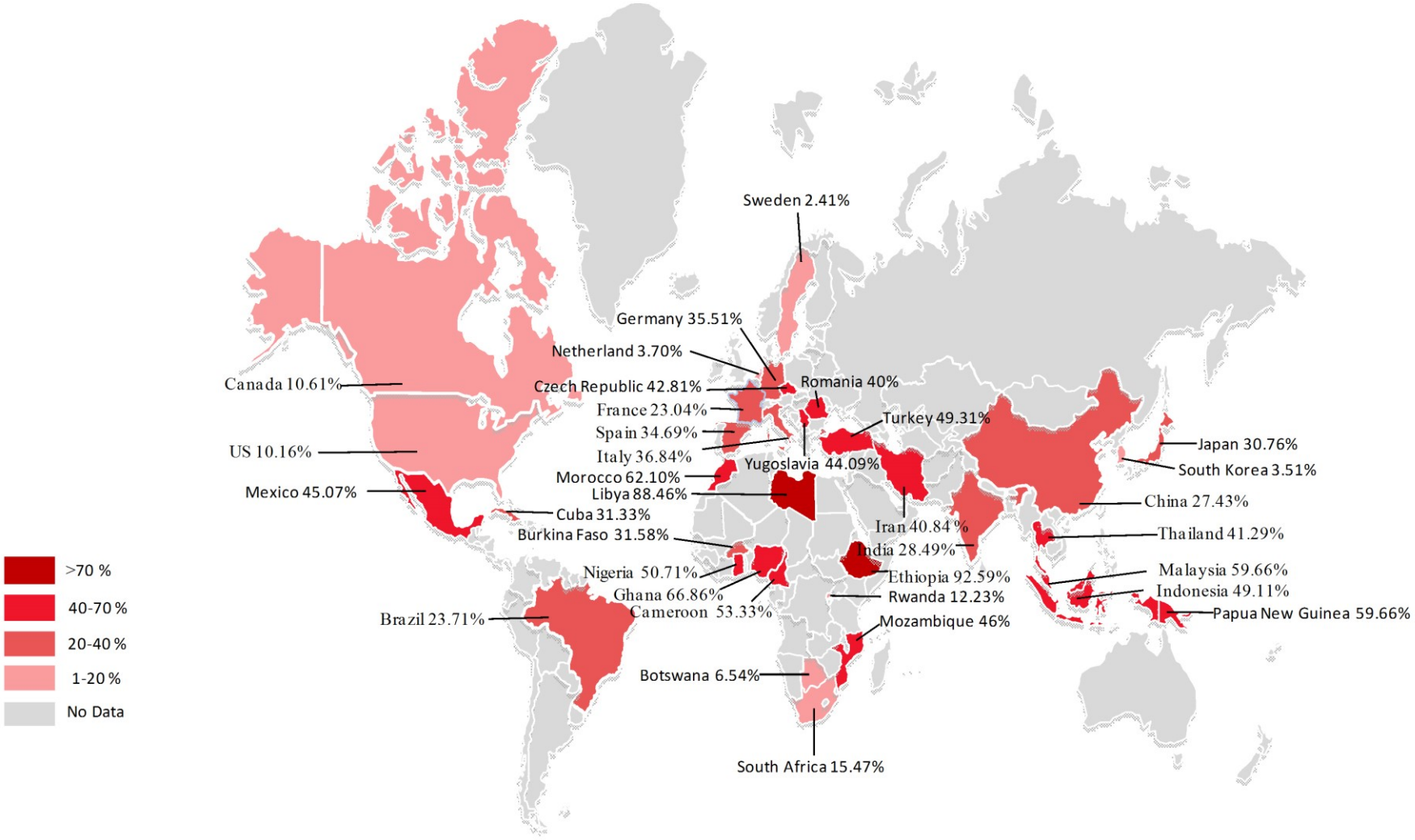
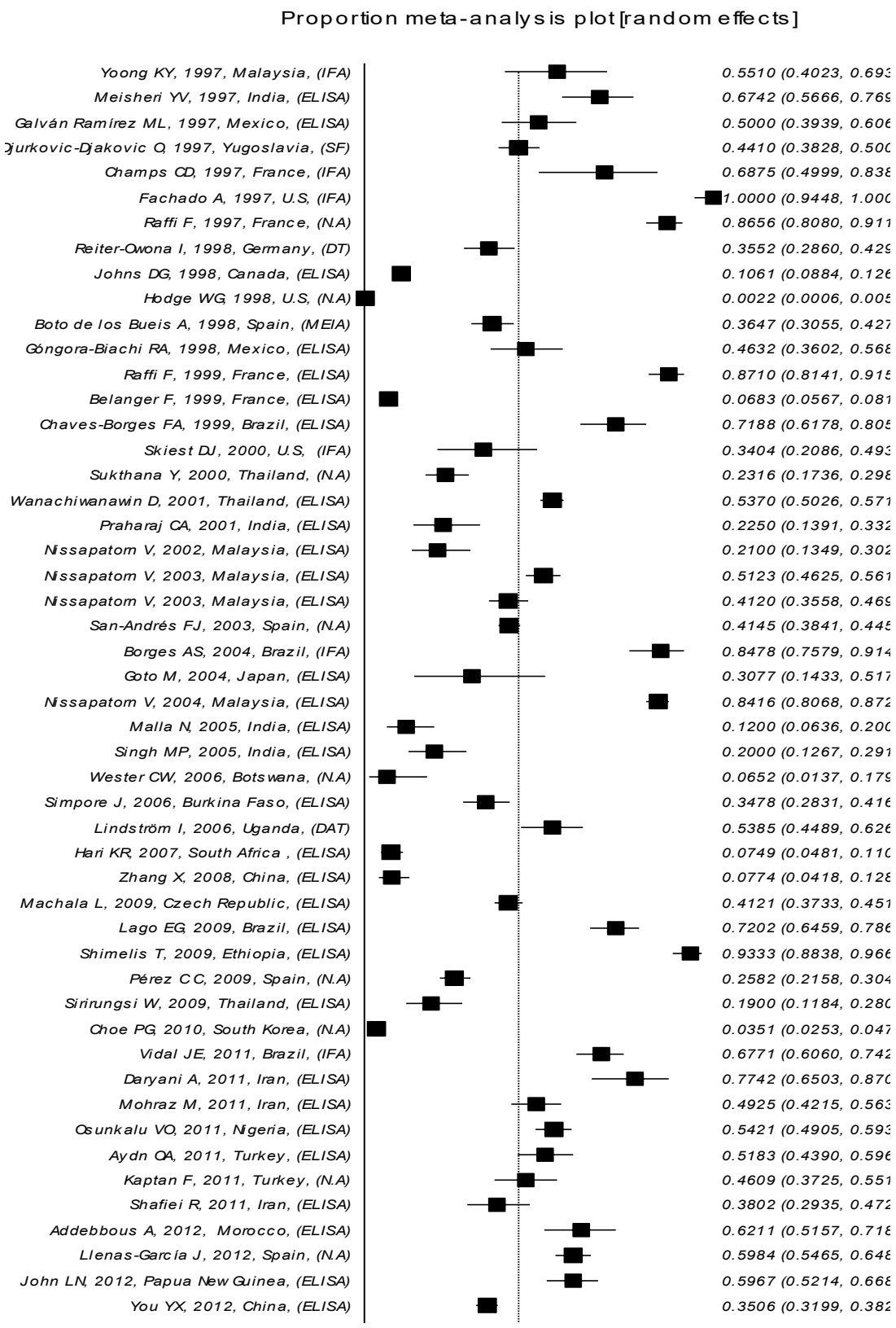
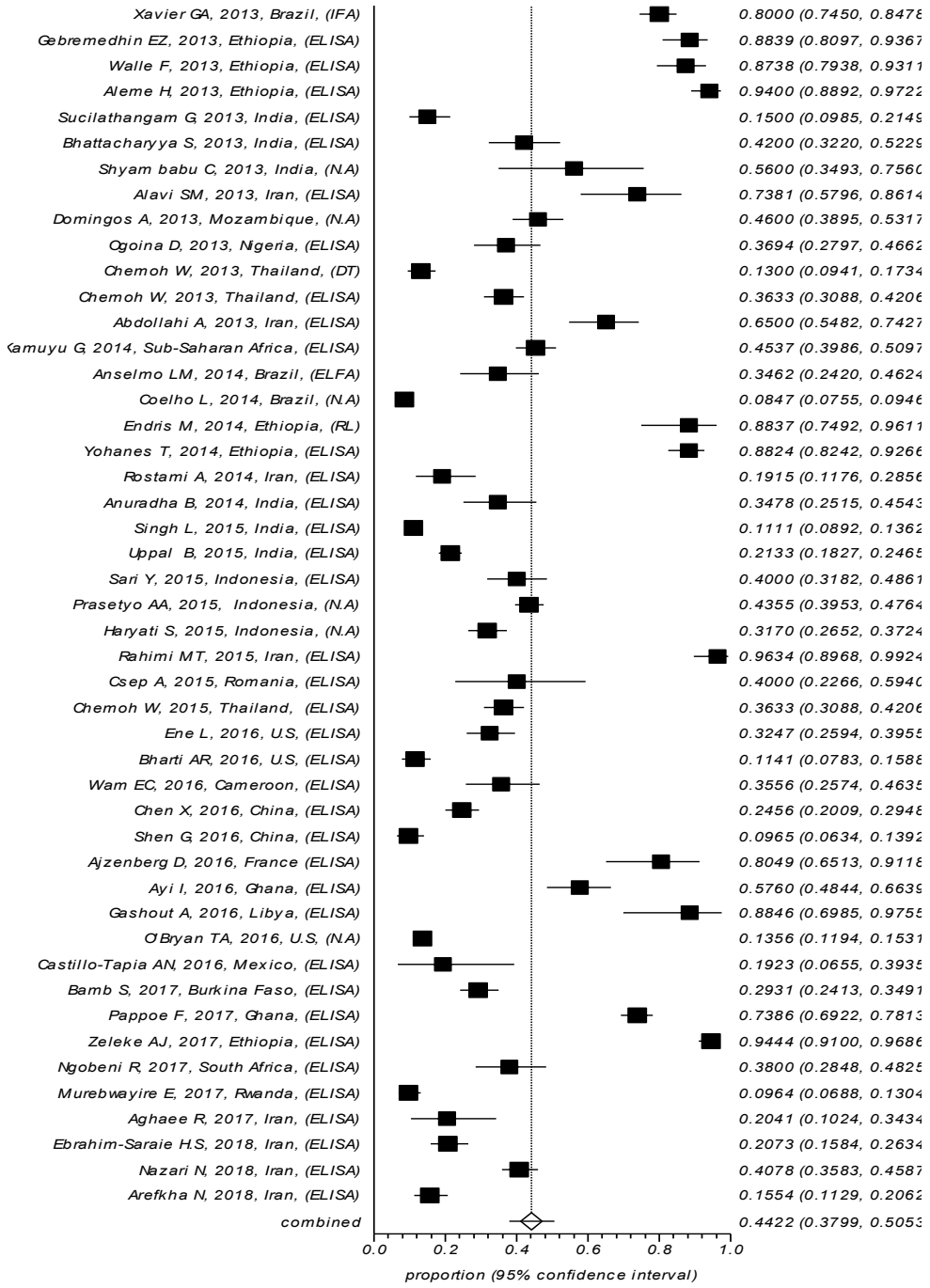
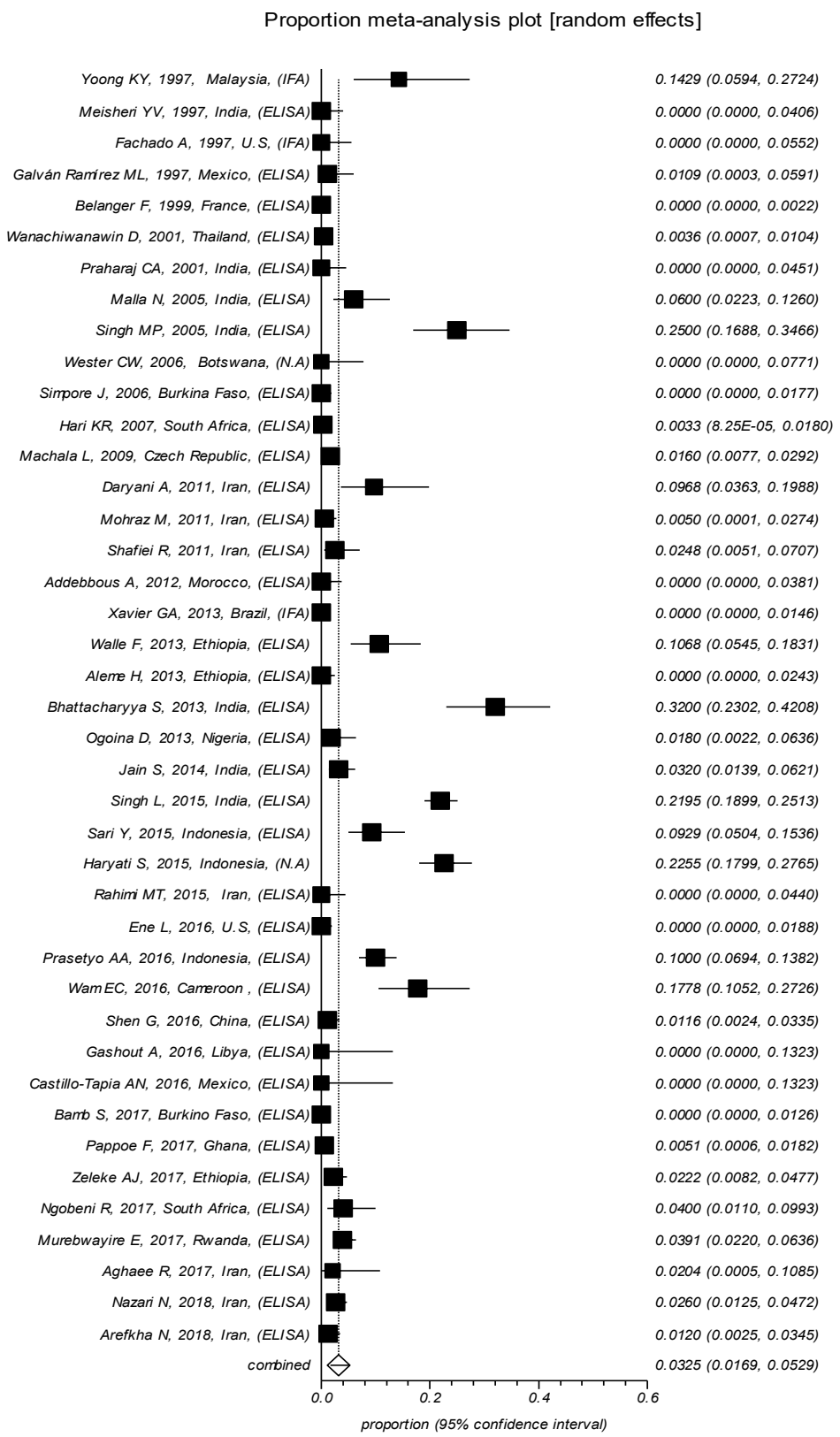


Figure 2. Overall *Toxoplasma* prevalence among PLHIV in different geographical regions.

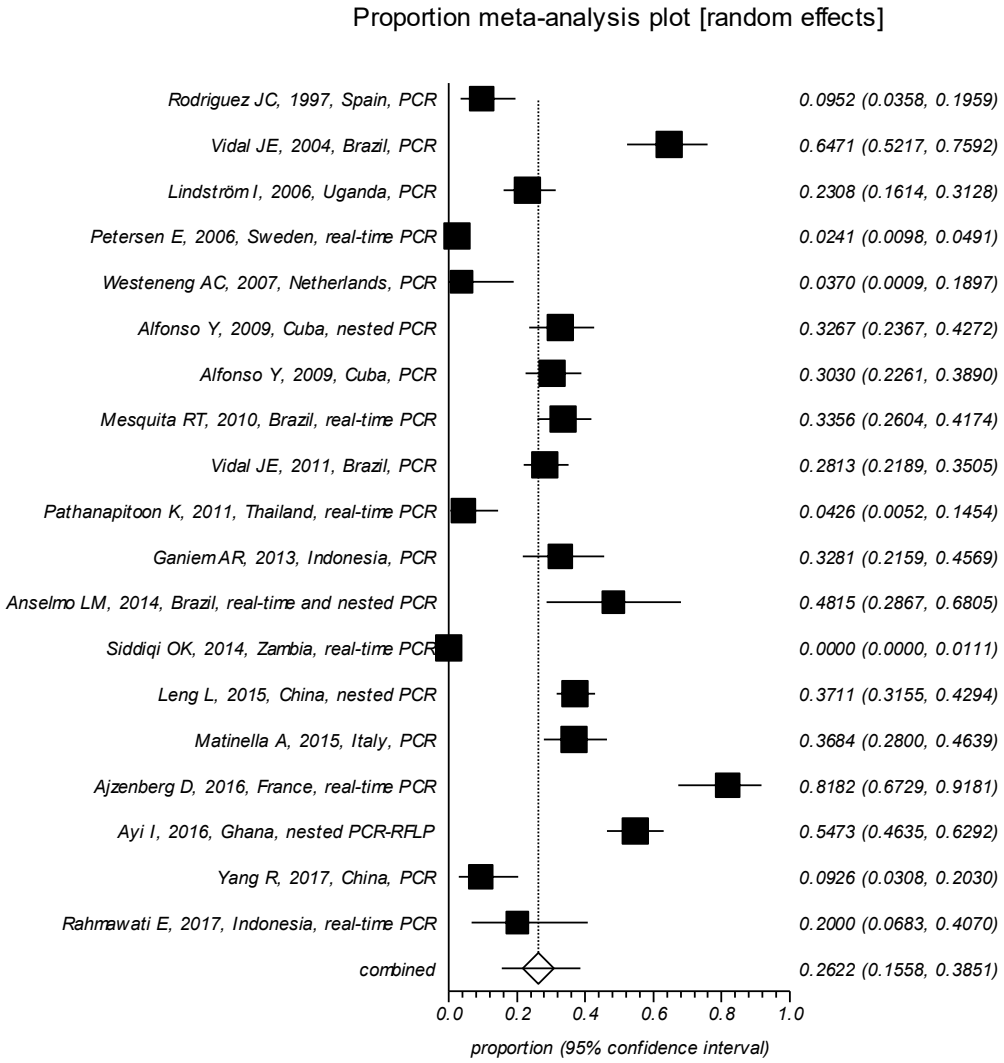

Proportion meta-analysis plot [random effects]
Yoong KY, 1997, Malaysia, (IFA) 0.5510 (0.4023, 0.693
Meisheri YV, 1997, India, (ELISA) 0.6742 (0.5666, 0.769
Galván Ramírez ML, 1997, Mexico, (ELISA) 0.5000 (0.3939, 0.606
Djurkovic-Djakovic O, 1997, Yugoslavia, (SF) 0.4410 (0.3828, 0.500
Champs CD, 1997, France, (IFA) 0.6875 (0.4999, 0.838
Fachado A, 1997, U.S, (IFA) 1.0000 (0.9448, 1.000
Raffi F, 1997, France, (N.A) 0.8656 (0.8080, 0.911
Reiter-Owona I, 1998, Germany, (DT) 0.3552 (0.2860, 0.429
Johns DG, 1998, Canada, (ELISA) 0.1061 (0.0884, 0.126
Hodge WG, 1998, U.S, (N.A) 0.0022 (0.0006, 0.005
Boto de los Bueis A, 1998, Spain, (MEIA) 0.3647 (0.3055, 0.427
Góngora-Biachi RA, 1998, Mexico, (ELISA) 0.4632 (0.3602, 0.568
Raffi F, 1999, France, (ELISA) 0.8710 (0.8141, 0.915
Belanger F, 1999, France, (ELISA) 0.0683 (0.0567, 0.081
Chaves-Borges FA, 1999, Brazil, (ELISA) 0.7188 (0.6178, 0.805
Skiest DJ, 2000, U.S, (IFA) 0.3404 (0.2086, 0.493
Sukthana Y, 2000, Thailand, (N.A) 0.2316 (0.1736, 0.298
Wanachiwanawin D, 2001, Thailand, (ELISA) 0.5370 (0.5026, 0.571
Praharaj CA, 2001, India, (ELISA) 0.2250 (0.1391, 0.332
Nissapatorn V, 2002, Malaysia, (ELISA) 0.2100 (0.1349, 0.302
Nissapatorn V, 2003, Malaysia, (ELISA) 0.5123 (0.4625, 0.561
Nissapatorn V, 2003, Malaysia, (ELISA) 0.4120 (0.3558, 0.469
San-Andrés FJ, 2003, Spain, (N.A) 0.4145 (0.3841, 0.445
Borges AS, 2004, Brazil, (IFA) 0.8478 (0.7579, 0.914
Goto M, 2004, Japan, (ELISA) 0.3077 (0.1433, 0.517
Nissapatorn V, 2004, Malaysia, (ELISA) 0.8416 (0.8068, 0.872
Malla N, 2005, India, (ELISA) 0.1200 (0.0636, 0.200
Singh MP, 2005, India, (ELISA) 0.2000 (0.1267, 0.291
Wester CW, 2006, Botswana, (N.A) 0.0652 (0.0137, 0.179
Simpore J, 2006, Burkina Faso, (ELISA) 0.3478 (0.2831, 0.416
Lindström I, 2006, Uganda, (DAT) 0.5385 (0.4489, 0.626
Hari KR, 2007, South Africa , (ELISA) 0.0749 (0.0481, 0.110
Zhang X, 2008, China, (ELISA) 0.0774 (0.0418, 0.128
Machala L, 2009, Czech Republic, (ELISA) 0.4121 (0.3733, 0.451
Lago EG, 2009, Brazil, (ELISA) 0.7202 (0.6459, 0.786
Shimelis T, 2009, Ethiopia, (ELISA) 0.9333 (0.8838, 0.966
Pérez CC, 2009, Spain, (N.A) 0.2582 (0.2158, 0.304
Sirirungsi W, 2009, Thailand, (ELISA) 0.1900 (0.1184, 0.280
Choe PG, 2010, South Korea, (N.A) 0.0351 (0.0253, 0.047
Vidal JE, 2011, Brazil, (IFA) 0.6771 (0.6060, 0.742
Daryani A, 2011, Iran, (ELISA) 0.7742 (0.6503, 0.870
Mohraz M, 2011, Iran, (ELISA) 0.4925 (0.4215, 0.563
Osunkalu VO, 2011, Nigeria, (ELISA) 0.5421 (0.4905, 0.593
Aydn OA, 2011, Turkey, (ELISA) 0.5183 (0.4390, 0.596
Kaptan F, 2011, Turkey, (N.A) 0.4609 (0.3725, 0.551
Shafiei R, 2011, Iran, (ELISA) 0.3802 (0.2935, 0.472
Addebbous A, 2012, Morocco, (ELISA) 0.6211 (0.5157, 0.718
Llenas-García J, 2012, Spain, (N.A) 0.5984 (0.5465, 0.648
John LN, 2012, Papua New Guinea, (ELISA) 0.5967 (0.5214, 0.668
You YX, 2012, China, (ELISA) 0.3506 (0.3199, 0.382



Supplementary Figure 1. Forest plot diagram of the studies reporting *Toxoplasma* IgG antibody among PLHIV



Supplementary Figure 2. Forest plot diagram of the studies reporting *Toxoplasma* IgM antibody among PLHIV



Supplementary Figure 3. Forest plot diagram of the studies reporting *Toxoplasma* infection based on molecular methods among PLHIV