Prevalence of Toxoplasmosis in Blood Donors: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Toxoplasmosis is a prevalent public health problem that infects humans and animals. Humans can become infected by consuming improperly washed vegetables, raw meat, contaminated water, or exposure to cats or their feces. Infection can also occur through the transfusion of contaminated blood. The current review aimed to assess the seroprevalence of Toxoplasmosis in blood donors globally.

Methods: After registering with PROSPERO (CRD42024597115), the search was carried out in databases including PubMed and Google Scholar from January 2014 to December 2024 in the English language using the PRISMA guidelines. A total of 15 cross-sectional studies reporting the prevalence of toxoplasmosis among blood donors, involving any serological method for detecting total antibodies or IgG only, having a sample size of more than 100, and free full text available, were included. The exclusion criteria included studies with incomplete data, non-cross-sectional designs, or duplicate reports. The I2 statistic was used to evaluate heterogeneity. The risk of bias was assessed on the Newcastle-Ottawa scale. The funnel plot was developed to assess publication bias.

Results: A total of 8610 participants were analysed in 15 included studies. The age range of included participants was 18 to 65 years. Out of 15, six studies were conducted in Africa, five in Asia, two in Europe, and one each in North America and transcontinental. The serological tests performed in all studies included enzyme-linked immunosorbent assay (ELISA), immunofluorescent assay, serum antibody detection, and latex or direct agglutination tests. The estimated pooled seroprevalence of Toxoplasmosis in blood donors was 32.6% (95% CI, 25.3–40.9%). The highest prevalence was in Asia (36.2%) and the lowest in America (13.5%). We found a significant association of prevalence with two risk factors: area of residence (OR = 1.61, p <0.001) and contact with cats (OR = 1.30, p = 0.002).

Discussion: A moderate seroprevalence of Toxoplasmosis infection was found in blood donors, showing a significant association with the residence of blood donors and a history of contact with cats. There is low heterogeneity among studies, stable sensitivity analysis results, and most of the included studies showed satisfactory risk of bias so this meta-analysis's precision is moderate to high.

Keywords: Meta-Analysis, Systematic Review, Seroprevalence, Toxoplasma Gondii, Toxoplasmosis.

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INTRODUCTION

Toxoplasmosis is a common parasitic infection resulting from Toxoplasma gondii, occurs worldwide[1]. Its prevalence varies, highest in Africa and Europe, with global seroprevalence between 30-50%[2,3]. The incubation period lasts from a week to a month, with symptoms ranging from none to flu-like effects or severe complications in immunocompromised patients, including abortion, congenital abnormalities, encephalitis, and death[4,5]. The primary spread is through the feco-oral route via contaminated water and food, but congenital and blood-borne transmission also occurs commonly[6,7,8,9].

Age, residence, eating habits, personal hygiene, low immunity, and animal contact are key risk factors for toxoplasmosis[10,11]. Screening for toxoplasmosis in blood donors is not practiced universally, with recipients often being immunocompromised, making them vulnerable to severe complications. Recent studies have investigated toxoplasmosis prevalence in blood donors[11,12,13]. There are different methods to determine the seroprevalence such as enzyme immunoassays and agglutination methods[14,15,16,17].

The rationale of this study was to synthesize epidemiological data on Toxoplasma gondii prevalence among blood donors globally, providing essential insights into transfusion-related risks and guiding screening policies and prevention measures. The aim of the study was to assess the global seroprevalence of toxoplasmosis while analyzing key risk factors, including gender, residence, raw meat consumption, cat exposure, and blood transfusion history. Our findings will empower health sector stakeholders and policymakers to enhance control measures for toxoplasmosis, significantly reducing its global impact.

METHODS

This systematic review and meta-analysis followed established recommendations and is presented following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42024597115).

Search strategy and study selection: Two databases (Google Scholar and PubMed) were searched systematically to find published cross-sectional studies that were relevant, published from January 2014 till December 2024 in English language with filter including free full text. The search strategy is presented in supplementary file. To enhance the quality of outputs, we thoroughly examined the reference lists of the studies included in our analysis to identify additional relevant studies. The search terms employed included: Toxoplasma, Toxoplasma gondii, T. gondii, Toxoplasmosis, seroprevalence, seropositive, blood donation, blood donors, transfusion with the Boolean operator "AND" and "OR". A third researcher examined the agreement and discrepancies present among the results.

Inclusion and Exclusion Criteria

Articles met these criteria for selection: (i) cross-sectional studies conducted between January 2014 and December 2024; (ii) involving blood donors as the target population; (iii) employing serological methods for total antibodies or solely IgG; (iv) indicating the frequency and specific count of seropositive donors; and (v) featuring a sample size exceeding 100. (vi) free full text available. Case reports, reviews, letter to editor, studies without prevalence of T. gondii antibodies, or irrelevant data were excluded from the current systematic review and meta-analysis. In the next step, duplicates were removed, the title and abstract of the remaining papers were assessed. The eligibility of the papers was assessed independently by two researchers, and any potential contradictions were resolved through consultation with the lead investigator.

Data Extraction

We imported the downloaded set of records from each database to the bibliographic software package EndNote X7 to remove duplicate records and facilitate retrieval of relevant articles. The required data was collected using a data extraction form that included the author's name, country, year of publication, sample size, number of seropositive cases, diagnostic method employed, and results through the evaluations of two reviewers based on the inclusion criteria. Any discrepancies were settled through discussions between the two reviewers, and if needed, the input of a third author was sought.

Risk of Bias Assessment

The New Castle Ottawa scale was used to assess the risk of bias in the included studies. It is an eight-item instrument assessing studies based on the selection of participants, comparability, and outcome. It provides a rating system ranging from 0 to 10 stars, where studies having scores ranging from 8-9 were considered as very good studies, 6-7 as good studies, 4-5 as satisfactory, and 0-4 as unsatisfactory studies[18]. Two independent reviewers assessed each study for eligibility and quality. Disagreements were resolved through discussion or by consulting a third reviewer.

Meta-Analysis

A random-effects model was employed for the meta-analysis. We calculated point estimates and 95% confidence intervals (CIs) for the pooled prevalence (PP) across individual studies. Utilizing Comprehensive Meta-Analysis software version 3.3,

we created a forest plot. The size of the square denotes the weight of each study, while the crossed lines indicate their respective confidence intervals. We assessed the heterogeneity of the studies using the I^2 statistic, with significance defined as greater than 50%. For subgroup analysis, studies were stratified based on sample size, diagnostic methods, and geographic regions, and the odds ratio was calculated for each risk factor. To illustrate publication bias, we used a funnel plot, and its statistical significance was evaluated through Egger's regression asymmetry test, with a p-value < 0.05 indicating potential small-study effects or publication bias. A sensitivity analysis was also performed by excluding studies with unsatisfactory scores to evaluate the robustness of the findings.

RESULTS

Characteristics of the Eligible Studies

The initial database search yielded 69 articles. After eliminating duplicates and irrelevant papers, 15 research articles were identified as eligible for inclusion in this systematic review and meta-analysis. **(Figure 1)**.

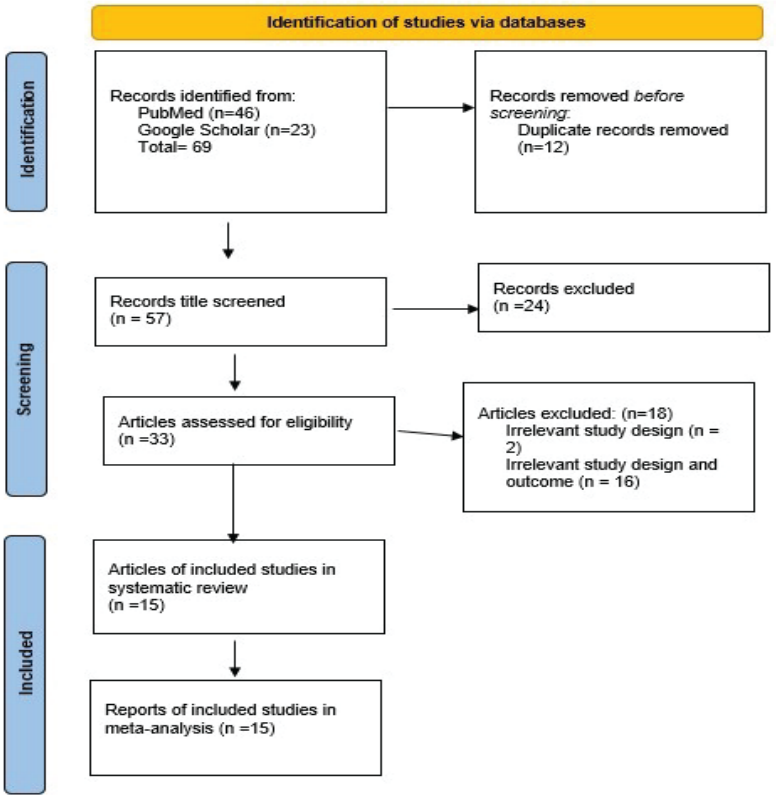

Figure 1: PRISMA Flowchart

Table 1: Baseline Characteristics of Included Studies!

Study, Year, and Country	Sample Size	Diagnostic Method	Sero Positivity	Data Of Donors (Gender, Age, Education Level, Occupation And BG)	Results
Abimbola, Nigeria, 2019[19]	248	Enzyme immunoassay	19.8 %	The age group 29–39 had the highest prevalence of anti-T. gondii at 58%, with 93% of those being males.	The prevalence of Toxoplasma among blood donors was notably high
Arwa, Tunisia, 2020[20]	800	Enzyme immunoassay	44.4%	The prevalence was 79.5%, with 636 out of 800 participants being male. The average age was 35 years, ranging from 18 to 62. The highest seroprevalence of infection, at 58.3%, was observed in the age group of 26 to 35 years.	Toxoplasma seropositivity exhibited a strong correlation with donor age.

Ayşegül Turkey 2020[21]	879	Enzyme immunoassay	25.6%	The participants comprised 90% males and 10% females, with an age range of 18 to 65 years and a mean age of 34 years.	Toxoplasmosis seropositivity is more prevalent in rural areas and the regions with education levels are low.
Bahador, Iran, 2014[22]	1480	Enzyme immunoassay	19.3%	Mean age = 39.1 (range: 20-68 years of age). Most of the subjects were aged 31-40 years. Males = 94.3% & females 5.7%. The seroprevalence rate of *Toxoplasma* was lower in educated people and this difference was statistically significant	The seroprevalence rate of *Toxoplasma* infection in blood donors is high
Cosme Alvarado, Mexico, 2016[23]	408	Enzyme immunoassay	13.5%	The mean age was 31.77 years (± 9.52), with a range of 18 to 60 years old. Of the participants, 55 (13.5%) tested positive for anti-T. gondii IgG antibodies.	Seropositivity of toxoplasma is associated with obstetric events and age.
Khaled A, Libya, 2021[24]	164	Antibody detection	33.5 %	Mean age = 35. Of all donors were males 46.3 % were unemployed or farmers and 17.1% employed were	*Toxoplasmosis* is prevalent among healthy blood donors in Tripoli health centers.
KalantariN, Iran, 2018[25]	500	Enzyme immunoassay	IgG 63.2%	Mean age = 25.16. 97.6%) were male.	The prevalence of toxoplasmosis is high in young healthy blood donors in north Iran.
Liliane, Abobo, 2016[26]	106	Enzyme immunoassay	IgG 64.15%	The average donor age was 31 years, ranging from a minimum of 20 to a maximum of 51 years. In	Increased endemicity found in blood donors.
Marwa, Egypt, 2024[29]	420	Enzyme immunoassay	16.4%	Average age: 32; 97.1% men. 66.2% highly educated volunteers. Low T. gondii infection in blood donors.	A low occurrence of T. gondii infection was observed in blood donors.
Milena, Serbia, 2022[30]	1095	Agglutination methods	20.5%	Gender distribution is almost equal	Blood donors exhibited a low prevalence of toxoplasmosis.
Mohammad Jafari, Iran, 2014[31]	375	Enzyme immunoassay	IgG 25%	Male: 88.29%, Female: 11.71%. The highest Toxoplasma seroprevalence (38%) was in the 25-36 age group; lowest (20.7%) in 17-25. No association was found between participants' occupation and Toxoplasma seropositivity (P > 0.05).	Results showed that 25% of blood donors had Toxoplasma infections before sampling, which can relapse with decreased immunity.
Reza, Iran, 2017[32]	194	Enzyme immunoassay	IgG 38.66%	The majority are males (96.9%), with an average age of 37.04 years.	*T. gondii* infections are very common among healthy blood donors in northwestern Iran.

Saeed, Iran, 2017[33]	491	Enzyme immunoassay	40.7%	The average age of participants was 36.29±4.16 years (with ages ranging from 18 to 57). The majority were male, constituting 93.9%.	*T.* gondii infections were found in healthy blood donors in northeastern Iran.

The baseline characteristics of included studies in terms of first author's name, publication year, country name, sample size, diagnostic method, seropositivity, and the study results are shown in **(Table 1).**

Across all included studies, a total of 8,610 blood donors were analyzed, with a pooled Toxoplasmosis prevalence of 32.6% (95% CI: 25.3–40.9%) based on IgG, total or antibody detection **(Figure 2).** Various modalities used for detecting seropositivity include ELISA, immunoassays, antibody detection, and agglutination assay. The heterogeneity assessed by I^2 statistic showed a value of 12.14 which indicates a low level of heterogeneity.

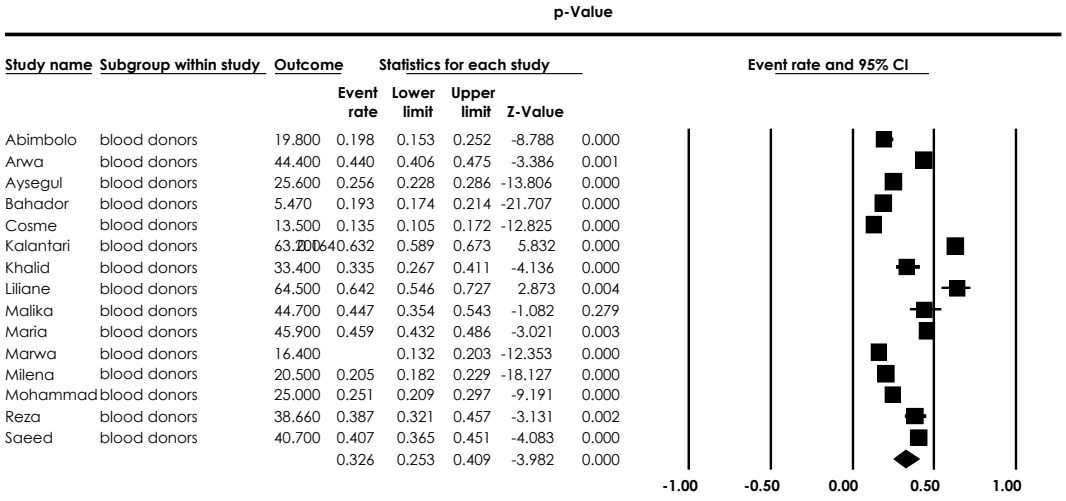

Figure 2: Forest Plot Diagram Based on Pooled Prevalence of Toxoplasmosis

Table 2: Pooled Prevalence on Basis of Subgroups

Subgroups	No. of Studies	Pooled Seroprevalence (95% CI)	P-Value	Heterogeneity (I^2)	References
Sample Size					
<500	9	31 (22.1-41.5)	0.001	17.672	19, 23, 24, 26, 27, 29, 31, 32, 33
500-1000	3	43.6 (24.7-64.6)	0.558	10.759	20, 21, 25
>1000	3	27.2 (13.6-47.1)	0.026	<0.001	22, 28, 30
Continent					
Africa	6	35.2 (22.9- 50.1)	0.052	13.413	19, 20, 24, 26, 27, 29
Asia	5	36.2 (20.9- 55.0)	0.148	<0.001	22, 25, 31, 32, 33
Europe	2	31.9 (12.7- 60.1)	0.202	<0.001	28, 30
North America	1	13.5 (10.5-17.2)	<0.001	<0.001	23
Transcontinental	1	25.6 (22.8-28.6)	<0.001	<0.001	21
Diagnostic Method					
Various Agglutination Methods	2	31.9 (12.7- 60.1)	<0.001	<0.001	28, 30
ELISA/ Immunoassays	12	32.6 (24-42.7)	0.202	12.938	19, 20, 21, 22, 23, 25, 26, 27, 29, 31, 32, 33
Antibody Detection	1	33.5 (26.7- 41.1)	0.001	<0.001	24

Concerning subgroups, studies were categorized according to sample size, continent of study, and the diagnostic test utilized for seropositivity assessment. Among the included studies, nine studies had a sample size of fewer than 500, with 31% of individuals seropositive for Toxoplasma antibody (p-value=0.001). Three studies were done on a sample size ranging from 500 to 1,000, showing 43.6% pooled prevalence among blood donors (p-value = 0.558). Three included studies had a sample size of more than 1000 with 27.2% of participants seropositive (p-value=0.026). On subgroups according to the continent of study, pooled seropositivity was 35.2% (p-value = 0.052) in Africa, 36.2% (p-value = 0.148) in Asia, 31.9% (p-value = 0.202) in Europe, 13.5% (p-value <0.001) in America, and 25.6% (p-value <0.001) in Turkey, labeled as transcontinental. When the included studies were grouped according to various methods of seropositivity assessment, 33.5% (p-value <0.001), 31.9% (p-value = 0.202), and 32.6% (p-value = 0.001) were seropositive on antibody detection, agglutination, and enzyme assays respectively **(Table 2).**

Table 3: Pooled Prevalence of *Toxoplasma Gondii* Infections in Relation to Various Risk Factors

Variables	No. of Studies	Sample Size	Cases (Percentage%)	Pooled Seroprevalence (95% CI)	Odds Ratio	P-Value
Gender						
Male	12	5488	1769 (32.23%)	33.5% (32.2-34.8)	0.977	0.833
Female	12	1636	543 (33.19%)	35.2% (32.8-37.7		
Residence						
Rural	7	908	468 (51.54%)	51.3% (47.9-54.7%)	1.615	<0.001
Urban	7	3931	1297 (32.99%)	34.8% (33.3-36.4%)		
History of Blood Transfusion						
Yes	2	45	25 (55.55%)	55.6% (40.7-69.6%)	0.987	0.968
No	2	946	476 (50.31%)	50.3% (47-53.6%)		
Consumption of Raw Meat						
Yes	7	1525	538 (35.27%)	36.3% (33.8-38.9%)	1.199	0.193
No	7	3125	1290 (41.28%)	43.1% (41.2-45.1%)		
Contact with cats						
Yes	5	785	312 (39.74%)	40.8% (37.3%–44.5%)	1.306	0.002
No	5	3142	1145 (36.44%)	37.5% (35.8-39.3%)		

Table 3 showed the results based on risk factors, included studies assessed the association between seropositivity and gender (n = 12), residence (n =7), history of blood transfusion (n = 2), consumption of raw meat (n = 7), and contact with cat (n = 5).

Figure 3 showed that a total of 5488 males and 1636 females were assessed in terms of *T. gondii* exposure in 12 studies, among which 1769 males [33.5% (95% CI: 32.2-34.8)] and 543 females [35.2% (95% CI: 32.8-37.7)] were found to be seropositive (OR = 0.977; 95% CI: 0.784–1.217; *p*= 0.833).

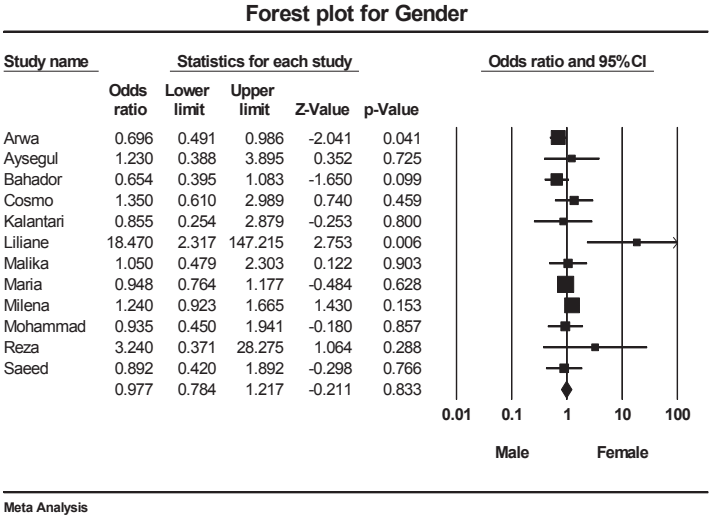

Figure 3: Forest Plots for Gender

Figure 4 showed that Seropositivity noted among blood donors in rural areas was 51.3% (95%: 47.9-54.7) showing

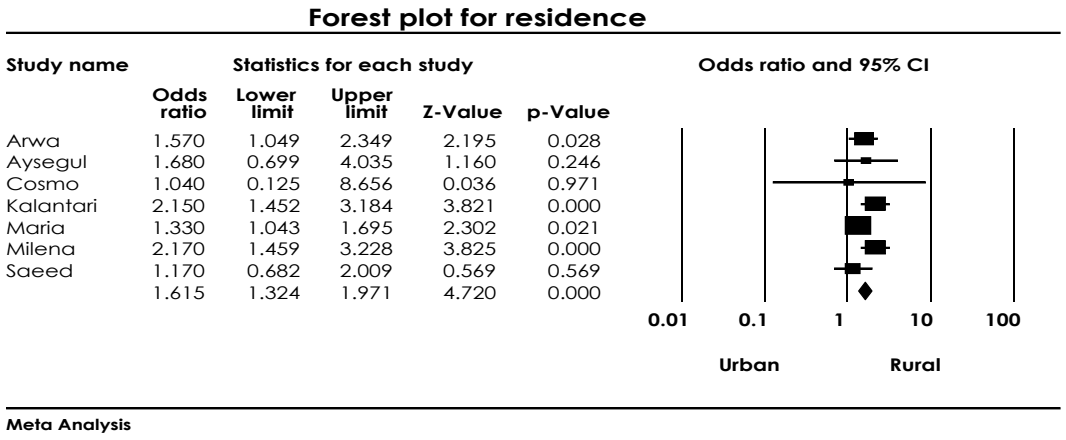

Figure 4: Forest Plots for Residence

Figure 5 showed that Consumption of raw meat was assessed among 4650 individuals, among which 1525 gave a positive answer and 538 of them showed seropositivity with an insignificant association [36.3% (95% CI: 33.8%–38.9%)] (OR = 1.199; 95% CI: 0.913–1.575; p= 0.193).

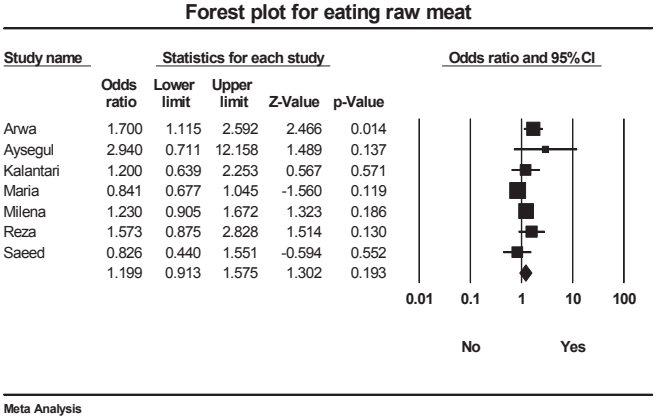

Figure 5: Forest Plots for Eating Raw Meat

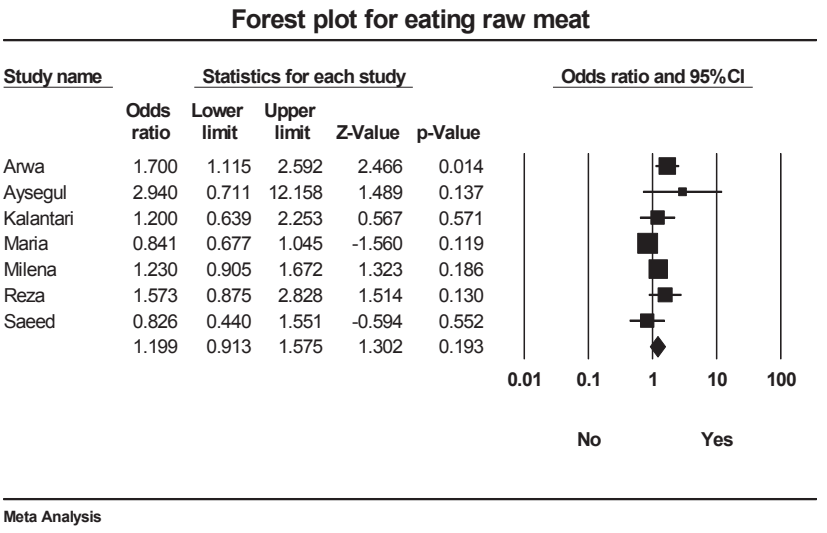

Figure 6 showed that among the included studies, 312 out of 785 individuals who had a history of contact with cats showed seropositivity of 40.8% (95% CI: 37.3%–44.5%) and significant association (OR = 1.306; 95% CI: 1.100–1.551; *p* = 0.002).

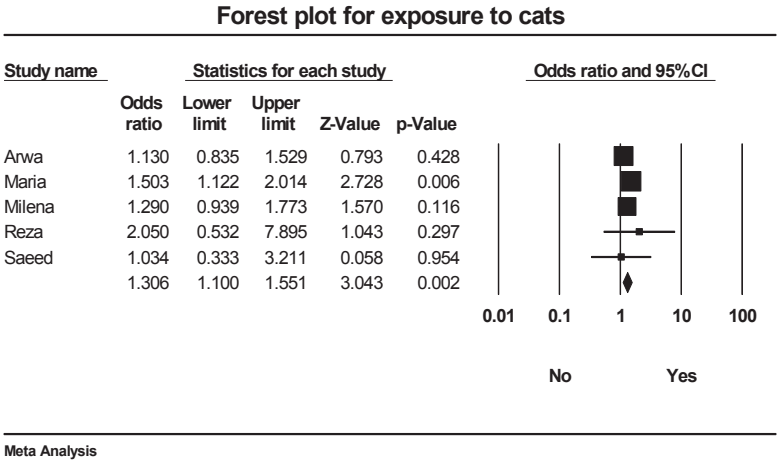

Figure 6: Forest Plots for Exposure with Cats

Figure 7 showed that the pooled prevalence of toxoplasmosis among patients with a history of blood transfusion was 55.6% (95% CI: 40.7-69.6) with no association [OR= 0.987 (95% CI: 0.531-1.835) p=0.968].

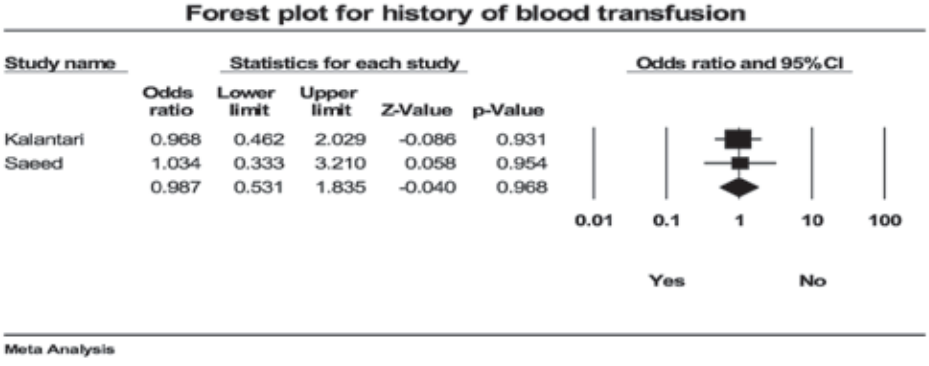

Figure 7: Forest Plots for History of Blood Transfusion

Risk of Bias

Table 4: Risk of Bias of Included Studies

Study, Year and Country	Selection (Maximum of 5 Stars)	Comparability (Maximum Of 1 Star)	Outcome (Maximum 3 of Stars)	Total	Conclusion
Abimbola, Nigeria, 2019	***	*	**	6	Good
Arwa, Tunisia, 2020	****	*	*	6	Good
Ayşegül, Turkey, 2020	***	*	**	6	Good
Bahador, Iran, 2014	***	*	***	7	Good
Cosme Alvarado, Mexico, 2016	**	*	***	6	Good
Khaled A. Libya, 2021	***	0	**	5	Satisfactory
Kalantari, N. Iran, 2018	***	0	***	6	Good
Liliane, Abobo 2016	***	0	*	4	Satisfactory
Malika, Algeria 2022	**	*	**	5	Satisfactory
Maria, Romania 2022	****	*	***	8	Very good
Marwa, Egypt 2024	***	*	*	5	Satisfactory
Milena, Serbia 2022	***	*	*	5	Satisfactory
Mohammad Jafari Iran 2014	***	*	*	5	Satisfactory
Reza, 2017, Iran	***	*	*	5	Satisfactory
Saeed Iran 2017	***	*	*	5	Satisfactory

The risk of bias for included studies was assessed using adapted New-Castle Ottawa Scale for Cross-sectional Studies where the score of very good studies range from 8-9 points, 6-7 points for good studies, 4-5 points for satisfactory studies and 0-4 points for unsatisfactory studies. Out of 15 included studies, one of the studies graded as very good scoring 8, [28] six were assessed as good studies scoring 6-7 points on scale[19,20,21,22,23,25]. A total of 8 studies were graded as satisfactory studies scoring 5-6 points[24,26,27,29,30,31,32,33]. **(Table 4)**

Sensitivity Analysis

When sensitivity analysis was performed by removing the study of unsatisfactory category, the estimated pooled prevalence [30.7% (23.5%-38.9%)] was slightly different from our main results [32.6% (95% CI: 25.3–40.9%)].

Publication Bias

The funnel plot showed asymmetrically distributed studies, with more studies on the left side of the plot compared to the right suggesting a potential publication bias (smaller studies with less significant results are missing). **(Figure 8)** The statistical significance was assessed by Egger's regression asymmetry test. Since the confidence interval (-12.72-5.74) included zero and the p-value (0.76) was much greater than 0.05, there was no significant evidence of publication bias.

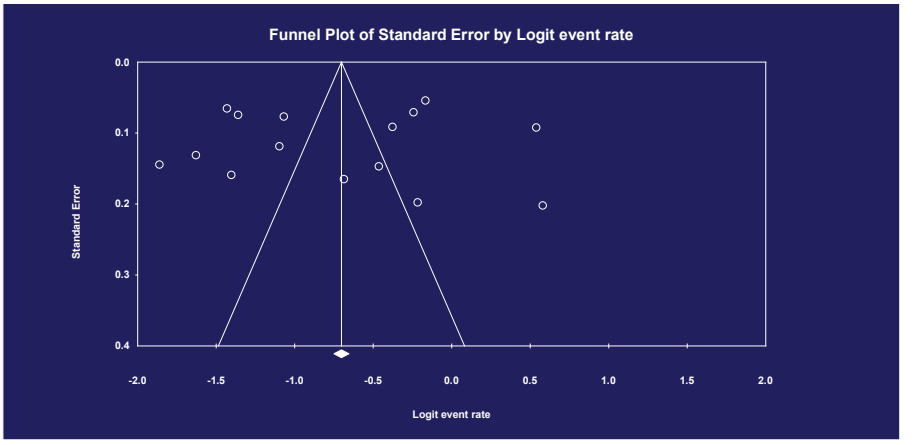

Figure 8: Publication Bias of The Included Studies

Table 5: Assessment of Precision Using GRADE Criteria

Criteria	Findings	Impact On Precision
Effect Size & CI	32.6% (95% CI: 25.3%–40.9%)	Moderate (CI width = 15.6%)
Sample Size	8,610 participants	Large sample = good precision
Heterogeneity (I^2)	12.14% (Low heterogeneity)	Improves precision
Publication Bias	Egger's test p = 0.76 (No bias detected)	No need to downgrade precision
Sensitivity Analysis	Effect size remains stable (30.7%)	Consistency supports precision

Due to the moderate confidence interval width, low heterogeneity, and consistent sensitivity analysis results, the precision of this meta-analysis is deemed moderate to high. No significant downgrades for precision are required in the GRADE assessment, reinforcing the reliability of the findings and enhancing confidence in the reported pooled prevalence estimate **(Table 5).**

DISCUSSION

The incidence of Toxoplasmosis infection is increasing and there is a rise in clinical cases in immunocompromised patients. Toxoplasmosis is a serious public health problem making its prevention and control essential. Toxoplasma is a pathogen that can be transmitted through transfusion. Our meta-analysis was done using 15 studies conducted during the last decade. We observed an overall PP of 32.6%, consistent with a review done in Nigeria of 32.92%[34]. Similar findings were noticed in a system-atic review done in Iran with an overall prevalence of infection 32.9%[35]. Our finding is however, higher than the PP of 6.26% reported in a meta-analysis done in China[36]. However, this difference may be due to better economic conditions and sanitary infrastructure in China. Our review results were in contrast to a meta-analysis done in Brazil showing the prevalence of 48 [37].

Most studies included in our review had sample sizes less than 500 showing PP of 31% while 3 studies had sample sizes between 500 and 1000 and their PP turned out to be 43.6%. Moreover, three studies had PP of 27.2% with sample sizes of more than 1000. In another systematic review done in Nigeria, studies were categorized into 4 groups according to sample size showed PP of 36.64%, 30.2%, 33.68%, and 32.59% in groups with sample sizes more than 450, 301-450, 151-300 and ≤150 respectively[34].

We categorized the included studies according to continent of study which showed the studies were conducted in Africa, Asia, Europe, and America. Only one study was conducted in Turkey which was considered as transcontinental. The PP based on continents were 35.2%, 36.2%, 31.9%, 13.5%, and 25.6% in Africa, Asia, Europe, America, and trans-continental, respectively. A documentary review investigating the endemicity of toxoplasmosis in blood donors showed seroprevalence of 40.7% in Africa, 7.8% in Asia, 38.1% in Europe, and 32.8% in America[38]. These prevalences are not consistent with the findings of our systematic review in the continents of Africa, Asia, and America. Another systematic review done for the assessment of toxoplasmosis prevalence in blood supplies and blood donors showed similar results in continents of Asia (29%) and Europe (30%). The result of this study was not comparable for the continents of Africa and America which showed higher PP of 46% and 42% respectively[39].

Our review included PP of toxoplasmosis based on the diagnostic methods utilized that showed 33.5%, 31.9% and 32.6% in antibody detection, various agglutination methods and enzyme immunoassays, respectively. A systematic review done in China showed seroprevalence of 7.30% with enzyme immunoassays and 3.16% with various agglutination methods in contrast to our results. This discrepancy may be due to lower overall PP in China.36 Similar to our results, PP for enzyme immunoassays was found in a study was 31.62% whereas PP for agglutination methods was considerably lower than our seroprevalence (14.41% vs 31.9%)[40].

The strength of our systematic review and meta-analysis included exhaustive database research including studies from all over the world, all studies had a cross-sectional design which helped to control various confounders and increased the validity of the review. Moreover, no statistically significant heterogeneity or publication bias was found among the studies. The precision of this meta-analysis is considered moderate to high in the GRADE assessment. This supports the robustness of the findings and strengthens confidence in the reported pooled prevalence estimate

Our review also had some limitations such as retrieval of only those studies freely available on databases. There was also a time limit of 10 years due to which we were unable to graph the trend of prevalence of toxoplasmosis over the past few decades. The authors could not search for more data sources that could retrieve a few more studies. The quality assessment by analyzing the risk of bias supported moderate confidence in the meta-analysis conclusions, though further high-quality studies could strengthen the evidence base.

CONCLUSION

Toxoplasmosis is prevalent in the whole world at moderate levels among blood donors. The study showed the overall prevalence of toxoplasmosis on the basis of gender, sample size, history of blood transfusion, exposure to cats, and intake of raw meat. To control the transmission of disease due to blood transfusion, blood donors should be screened rigorously for toxoplasmosis.

LIST OF ABBREVIATIONS

PP Pooled Prevalence
IgG Immunoglobulin
ELISA Enzyme-linked Immunosorbent Assay
OR Odds ratio
CI Confidence Interval

ACKNOWLEDGMENT

The authors wish to express gratitude to her parents and husband for their unwavering support.

CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTIONS

AIB and AR conceived and designed the study. AIB, AR, and FS conducted the literature search, identified relevant articles, screened them, and extracted the necessary data. AIB, AR, and LA carried out the statistical and meta-analyses and contributed to manuscript writing, while SQA and RM assisted in drafting the manuscript. All authors carefully reviewed and approved the final version of the manuscript.

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