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REVIEW

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Molecular and serological diagnosis of toxoplasmosis: a systematic review and meta-analysis

Isa Marianny Ferreira Nascimento Barbosa de Souza[1], **Victor da Silva Siqueira**[1], **Isabella da Costa Ribeiro**[2], **Laís Silva Pinto Moraes**[2], **Débora Pereira Gomes do Prado**[2], **Stéfanne Rodrigues Rezende**[2], **Webster Leonardo Guimarães da Costa**[1,3], **Hanstter Hallison Alves Rezende**[1]

ABSTRACT

Toxoplasmosis is an infection of vast worldwide distribution whose etiologic agent is *Toxoplasma gondii*. This disease can cause problems ranging from mild symptoms to serious conditions, such as encephalitis, miscarriage and blindness. Therefore, it is of utmost importance to perform a diagnosis with reproducible techniques in order to obtain a good prognosis. The aim of this review was to analyze the efficiency of toxoplasmosis diagnostic techniques based on sensitivity and specificity results. Five research platforms in English language were used (Eric, Elsevier, Google Scholar, PubMed and SciELO), which contained data on the diagnosis of toxoplasmosis. The search and selection were performed for studies published prior to June 2021. The search resulted in the inclusion of 13 articles published from 2005 to 2020. The data revealed the use of different samples in the standardization of techniques such as serum, total blood, colostrum and amniotic fluid. The flow cytometry, lateral flow immunoassay and qPCR techniques showed 100% sensitivity, whereas the ELISA, western blotting, qPCR and RE-LAMP techniques achieved 100% specificity. Significantly, the qPCR and LAMP techniques were more accurate when the likelihood ratio was assessed. The meta-analysis identified that ISAGA and western blotting have low sensitivity values and LIASON, ELFA and ELISA, using a silica bioconjugate, also have low specificity values. It was noted that a wide range of methods have high values of sensitivity and specificity. Therefore, the choice of the method will be based on the conditions and its financial viability.

KEYWORDS: Molecular diagnosis. Serology. *Toxoplasma gondii*. Zoonosis.

INTRODUCTION

The protozoan *Toxoplasma gondii* is the etiologic agent of toxoplasmosis, a zoonosis which is widely distributed around the world, and its prevalence ranges from 30% to 50% among the global population[1], varying according to the region, due to differences in climate, eating habits, hygiene and host susceptibility[2]. *T. gondii* infection is usually asymptomatic, but when it is symptomatic, it can cause a wide range of potentially fatal clinical symptoms, especially in cases of congenital infection and immunocompromised patients[3]. Regarding the laboratory diagnosis of toxoplasmosis, there are peculiarities that demand the use of different techniques to establish its confirmation. This occurs mainly in cases of ocular toxoplasmosis, since there is no consensus in the literature on how to diagnose the infection in pregnant women and immunocompromised patients[2-4].

[1]Universidade Federal de Jataí, Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Jataí, Goiás, Brazil

[2]Universidade Federal de Jataí, Curso de Biomedicina, Jataí, Goiás, Brazil

[3]Universidade Estadual de Campinas, Instituto de Biologia, Programa de Pós-Graduação em Genética e Biologia Molecular (Imunologia), Campinas, São Paulo, Brazil

Correspondence to: Hanstter Hallison Alves Rezende
Universidade Federal de Jataí, Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Jataí, GO, Brazil
Tel: +55 62 98222-2759

E-mail: hanstter@gmail.com

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Due to this diagnostic complexity, numerous laboratory techniques have been developed over the years. These techniques are classified as direct, involving the detection of *T. gondii* or its parts, or indirect, which involves the detection of cytokines or antibodies. It is important to emphasize that each technique has its particularities and indication, and diagnosing toxoplasmosis requires much more than the isolated analysis of each one of them. There is a need to assess the results in an integrated way and according to each form of clinical manifestation, respecting the likely time of infection and the limits of each of the techniques used[2,4,5].

Therefore, considering that to establish the diagnosis of toxoplasmosis there are different diagnostic techniques and each of them has its own characteristics and own indication, this review aims to analyze the techniques and efficiency markers for identifying *T. gondii* through the analysis of studies published in the literature.

MATERIALS AND METHODS

Protocols

The study was characterized by the analysis of articles published between 1942 and 2021 which addressed different diagnostic methods for toxoplasmosis, ways of interpreting data and evaluation of the antigens. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol was used.

Systematic strategy of search

The literature search was systematically conducted using five databases: Eric, Elsevier, Google Scholar, PubMed and SciELO. All scientific publications related to the diagnostic characteristics of *T. gondii* were searched. The search process was performed using synonyms and combinations of various search terms, including *Toxoplasma gondii* OR *T. gondii* OR toxoplasmosis OR neurotoxoplasmosis OR congenital toxoplasmosis AND diagnosis OR molecular diagnosis OR serology OR immunology AND human diagnosis OR woman diagnosis OR antibody detection. The search was restricted to the English language.

Study selection criteria

The studies were identified and selected independently by two researchers through the title and abstract, and those that met the inclusion criteria were considered eligible. The main inclusion criterion was the presence of different types of anti-*T. gondii* antibody diagnosis and specific antigen diagnosis such as proteins. Therefore, all publications that mentioned diagnostic methods using such markers were included in the study. It was observed that articles from 1942 to 1999 had old diagnostic techniques that would not be consistent with the research and these were excluded, along with studies that presented diagnostic techniques for different types of parasites, duplicate articles and articles that addressed transmission, infection treatments and toxoplasmosis in animals. Disagreements were resolved through discussion and consensus with a third researcher. As for the review articles, studies that had authorization for data reproduction were included.

Data extraction

From the selection of articles, the main data from the studies were independently extracted and summarized according to the PRISMA checklist. Afterwards, the general characteristics of the articles were obtained, including authors, year of publication and country where the study was carried out, as well as the methodological characteristics, such as techniques used for the diagnosis of toxoplasmosis, samples and respective markers. The sensitivity values were calculated using the probability of a positive result in patients and the specificity values were calculated using the probability values of true negatives.

Statistical analysis

The data obtained from the studies were submitted for statistical analysis using the Chi-square test for the likelihood ratio values and a parameter that assesses the precision or accuracy of the techniques, while the proportional meta-analysis was performed using the R® software (version 4.2.2., Ross Ihaka, Auckland, New Zealand) in order to verify the confidence intervals of the sensitivity and specificity values.

RESULTS

General characteristics of the study and total results

Initially, a total of 4,280 articles related to toxoplasmosis diagnostic techniques were identified in the literature research. After reading the abstracts, 13 studies were selected for systematic review. All articles used in the analysis were published in English from 2005 to 2020 onwards. Figure 1 elucidates this bibliographic search and selection process. Among the selected studies, the use of samples such as serum, whole blood, colostrum and amniotic fluid was observed, and these were associated with different methods of detection of *T. gondii* and its respective

markers. Table 1 presents the general characteristics of the selected articles[6-18].

Overall, the techniques performed for diagnosis were LIASON (1 study), Flow Cytometry (1 study), Platelia (1 study), ELISA (4 studies), ELFA (1 study), ISAGA (1 study), Western Blotting (WB, 2 studies), LFIA (1 study), LAMP (2 studies), PCR (1 study), and q-PCR (1 study).

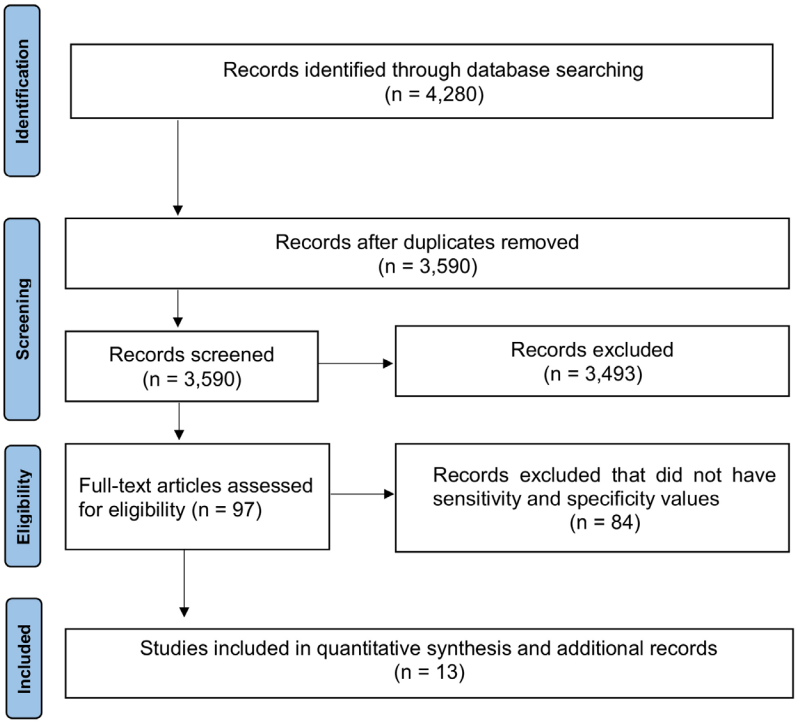

Figure 1 - Flow diagram of the literature research and selection of studies according to the PRISMA protocol.

Table 1 - General characteristics of the articles selected in the systematic review.

Article	Year	Country	Technique	Sample	Marker	Methodology
Petersen *et al*.[6]	2005	France	LIAISON	Serum	IgG	Chemiluminescence
Silva-dos-Santos *et al*.[7]	2012	Brazil	Flow cytometry	Serum	IgM and IgG	Fluorescence
Mouri *et al*.[8]	2015	France	Platelia	Serum	IgG and IgM	Enzyme Immunoassay
Oliveira *et al*.[9]	2015	Brazil	ELISA	Serum and colostrum	IgM and IgG	Enzyme Immunoassay
Drapala *et al*.[10]	2015	Poland	WB	Serum	P35, MAG1, MIC1 and ROP1	Molecular
Stajner *et al*.[11]	2016	Serbia	IgM-ISAGA	Amniotic fluid	IgM	Direct binding
Capobiango *et al*.[12]	2016	Brazil	WB	Serum	IgG	Molecular
Barros *et al*.[13]	2017	Brazil	ELISA and ELFA	Blood	IgM and IgG	Immunoassay and Serological
Aly *et al*.[14]	2018	Egypt	ELISA	Serum and urine	TLA	Immunoassay
Morovati *et al.*[15]	2019	Iran	LFIA	Serum	GRA-7	Immunochromatography
Luo *et al*.[16]	2019	China	ELISA	Serum	GRA2, GRA7 and TPI	Immunoassay
Soltani Tehraini *et al.*[17]	2020	Germany	qPCR and RE-LAMP	Blood	Rep529	Molecular
Hegazy *et al*.[18]	2020	Egypt	LAMP and PCR	Blood	Gene B1	Molecular

ELISA = Enzyme Immunosorbent Assay; WB = Western Blotting; ISAGA = Immunoabsorption and Agglutination Assay; qPCR = Real Time Polymerase Chain Reaction; LFIA = Lateral Flow Immunoassay; LAMP = Loop Mediated Isothermal Amplification; ELFA = Enzyme-Linked Fluorescent Immunoassay.

Sensitivity and specificity of *Toxoplasma gondii* diagnostic techniques

The sensitivity and specificity values of the diagnostic methods and their markers were extracted from each study and are described on Table 2. It was observed that the flow cytometry, lateral flow immunoassay and qPCR techniques had 100% sensitivity, whereas ELISA, western blotting, quantitative real-time (qPCR) and RE-LAMP techniques achieved 100% specificity. Added to this, the likelihood ratio values were calculated in order to determine which test would be accurate for the diagnosis. Significantly, qPCR and LAMP techniques were more accurate ($p < 0.05$).

Meta-analysis

The extracted data were submitted for meta-analysis in order to verify the confidence intervals of the methods. The sensitivity of each was analyzed in their respective proportional values, as shown in Figure 2. It was observed that the ISAGA techniques for IgA/IgM and western blotting IgG presented significantly low sensitivity with values outside the confidence interval. The technique with the highest sensitivity was the ELFA IgG.

Regarding the specificity values found in Figure 3, it was identified that the LIASON IgG/IgM, ELFA IgG/IgM and ELISA Bioconjugate techniques obtained significantly lower values. Overall, most methods showed good specificity, with values close to the confidence interval.

DISCUSSION

The selected articles allowed us to evaluate the efficiency of the techniques used in the diagnosis of toxoplasmosis. The systematic review showed that different samples such as serum, colostrum total blood and amniotic fluid were used in the development of identification methods for *T. gondii*. Furthermore, the methodologies vary, as both serological and molecular techniques are developed in the scientific sphere. This diversity of methods prompts greater reliability of the results, as serological techniques are supplemented by molecular techniques[19].

The data obtained demonstrated that the flow cytometry techniques with IgM and IgG, IFLA with GRA-7 and

Table 2 - Sensitivity and specificity values of the techniques used in the articles.

Technique	Marker	Sensitivity (%)	Specificity (%)	Likelihood ratio
LIASON	IgG/IgM	99.3	62.5	2.61
Flow Cytometry	IgG/IgM	100	90	10
Platelia	IgG	33.1	99.2	0.41
ELISA	IgG	94.2	100	17.24
ELISA	IgM	94.2	100	17.24
ELISA	IgA	94.1	100	16.94
ELISA	P35-MAG1	100	95	20.00
ELISA	MIC1-ROP1	77.3	95	14.60
ELISA	MAG1-ROP1	86.4	95	17.28
IgM-ISAGA	IgA	56.3	80	2.815
IgM-ISAGA	IgM	37.5	97.4	14.42
WB	IgG	64.3	100	2.80
qPCR	Gene B1	80	100	5.00
ELFA	IgG	92	80	4.60
ELFA	IgM	92	80	4.60
ELISA	Silica bioconjugate	90	80	4.50
LFIA	GRA-7	100	96.7	33.33
qPCR	GRA-2, GRA-7 e TPI	96.7	100	30.30
qPCR	Rep-529	100	94.1	**1.07***
RE-LAMP	Rep-529	71.42	100	3.49
LAMP	Gene B1	88.88	50	**1.77***

ELISA = Enzyme Immunosorbent Assay; WB = Western Blotting; ISAGA = Immunoabsorption and Agglutination Assay; qPCR = Real Time Polymerase Chain Reaction; LFIA = Lateral Flow Immunoassay; LAMP = Loop Mediated Isothermal Amplification; ELFA = Enzyme-Linked Fluorescent Immunoassay.

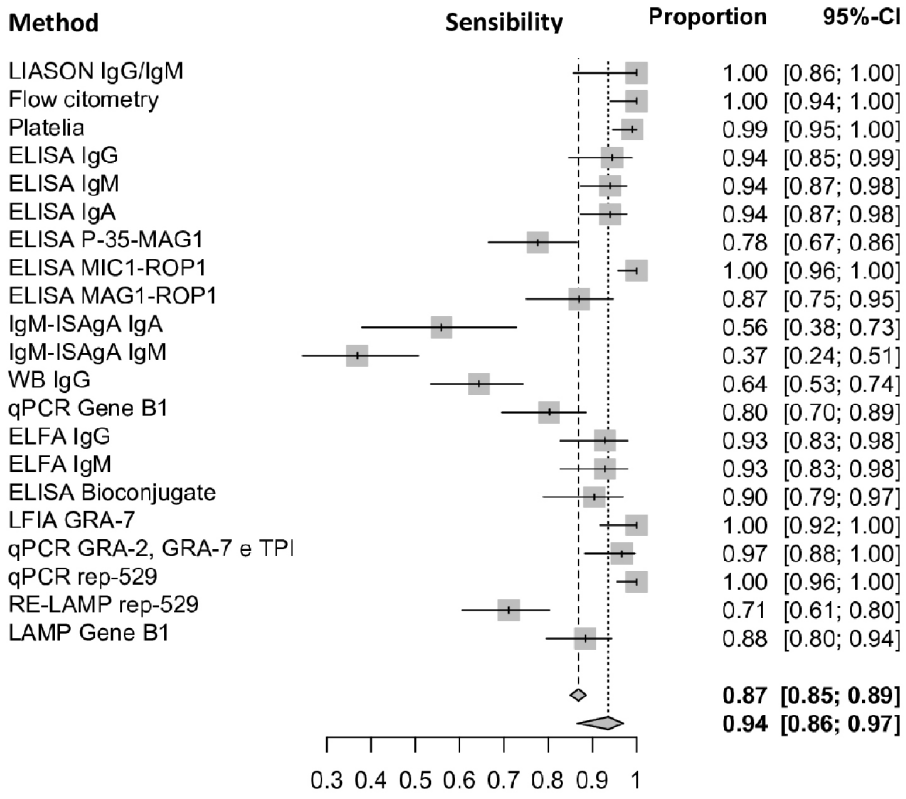

Figure 2 - Forest plot of the sensitivity values of serological and molecular techniques.

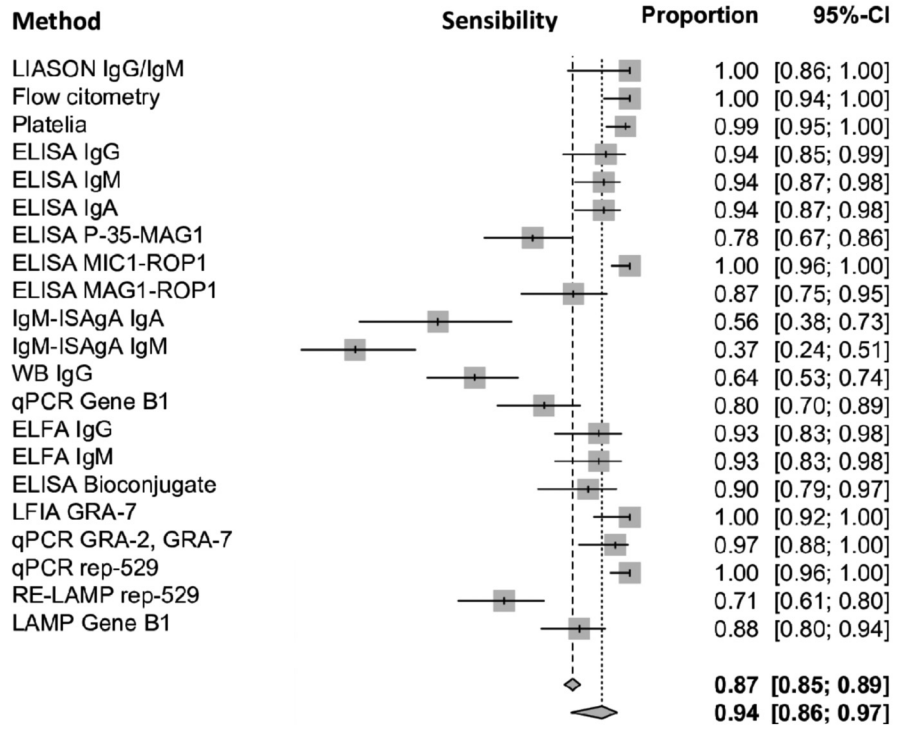

Figure 3 - Forest plot of the specificity values of serological and molecular techniques.

qPCR with rep 529 achieved high sensitivity values. Among these, flow cytometry has made great advances in the last 30 years, being considered a widely useful tool in the fields of immunology, molecular biology, bacteriology, virology, cancer biology and parasitology. The flow cytometer (fluorescent-assay activated cell sorting – FACS)

is also used in studies to monitor immune response against infectious agents such as *T. gondii*[20,21].

The lateral flow immunoassay technique showed 100% sensitivity. This result is due to the usage of recombinant antigens bound to colloidal gold. Since it presents instant results, in addition to allowing the detection of the parasite in both environmental and human samples, this method can be associated with molecular techniques and ensure an accurate diagnosis of toxoplasmosis[22].

Among the molecular techniques used in the detection of *T. gondii*, qPCR showed the best result, considering its diagnostic accuracy. This high performance identified is due to the fact that qPCR uses a fluorescent system to detect light from the gene amplification reaction at the same time as the reaction occurs[23]. The methodology allows greater agility, precision, reproducibility and accuracy. Furthermore, the final analysis of the reaction is done without the need for direct tube manipulation, which reduces the risk of contamination, false positive results and false negative results. Therefore, based on the above, it is clear that qPCR is the molecular technique with the best diagnostic performance, but due to its high cost, its application on a large scale becomes unfeasible in most laboratories[24,25].

Another technique that showed greater precision was the loop-mediated isothermal amplification (LAMP), which was developed by Notomi *et al*.[26]. This technique is based on the use of a constant temperature, in which it is possible to obtain an amount of amplified DNA on an exponential scale, being fast, efficient and highly specific. The Bst DNA polymerase enzyme, extracted from *Bacillus stearothermophilus* with strand displacement activity, is used for amplification. Amplification can be obtained in 15 to 60 min at a constant temperature between 60 and 65 °C. The advantages of the LAMP technique include the amplification of DNA under isothermal conditions and starting from few copies, as for PCR. It also has high specificity, it is easy to perform, and the qualitative result can be visualized with the addition of intercalants[27].

The confirmation of toxoplasmosis is mainly based on the detection of IgM and IgG antibodies. However, the investigation of IgA is also performed, especially when there is a need to determine the stage of infection. The immunoabsorption agglutination reaction (ISAGA) is a test used to detect IgM and IgA antibodies, as it has a specificity greater than 97%[28].

In the meta-analysis, it was found that the ISAGA technique had low sensitivity, being used for the identification of *T. gondii* in the amniotic fluid. The results of this method are revealed from the direct agglutination of the suspension of tachyzoites. Regarding specificity, it was shown to be a very specific test for anti-*T. gondii* IgM. However, it is hardly available for clinical use due to the difficulty of detecting positive results[29].

The IgG Western Blot (IgG-WB), which also showed low sensitivity, is a technique based on the separation of proteins by molecular weight, using electrophoresis, and the detection of the protein of interest through specific antibodies. It is used for confirmatory diagnosis of many diseases[30]. In toxoplasmosis, this technique demonstrates the presence of antigenic markers that enable distinguishing between the acute and chronic phase of the infection[31]. In recent years, it has been applied to confirm congenital toxoplasmosis with a sensitivity of 72 to 85% in the third month of life and, when combined with serology, the sensitivity increases to 94% with a specificity of 100%. Therefore, IgG-WB can be used for early diagnosis in combination with other markers of the infection, such as anti-*T. gondii* IgM and rep 529[32].

The enzyme-linked fluorescent immunoassay (ELFA) technique consists of the association of the immunoenzymatic method with a final fluorescence detection. According to the selected articles, it was possible to identify a sensitivity of 92% and a specificity of 80% for the IgG marker, being the technique with the most accurate sensitivity. This accuracy of sensitivity is due to the fact that the technique allows the detection of low levels of antibodies for long periods after the acute phase. In addition, high titers do not predict a recent infection in isolation. Thus, the technique has good specificity and sensitivity for IgG antibodies, being an effective method in the diagnosis of toxoplasmosis[32]. On the other hand, in a study with 76 pregnant women in the second and third gestational trimester, 49 had low IgM indices, shown by the ELFA technique as well as the result found in our meta-analysis[32,33].

CONCLUSION

From this study, it was possible to evaluate the effectiveness of different techniques and markers used in the diagnosis of toxoplasmosis. It was noticed that a wide range of methods have high sensitivity and specificity, so the choice of the method used will be based on the conditions and its financial feasibility. For example, qPCR using the marker rep 529 has been shown to be highly accurate, but it is expensive.

Furthermore, some techniques that have good sensitivity do not have high specificity and vice versa. This refers to the fact that the choice of technique is closely linked to its purpose. In scientific research, the preference is to use more specific methods, while in blood bank screening, a more sensitive method is sought.

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