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Review article

Saliva and tear as useful tools for the diagnosis of *Toxoplasma gondii* in human specimens: a systematic review

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ABSTRACT. Toxoplasmosis is diagnosed by serology, mainly using invasive specimens such as serum or cerebrospinal fluid. This study aimed to investigate whether saliva and tear samples can be used instead of serum samples to diagnose *Toxoplasma gondii*. Five English-language databases were checked up to 2021. Other types of non-invasive samples (milk and urine) were excluded from this study. In 15 cross-sectional studies, a total of 4338 saliva samples were examined, out of which 718/3848 (18.66%), 30/200 (15%), and 60/122 (49.18%) samples were positive for anti-*T. gondii* IgG, IgM, and IgA antibodies, respectively. And for tear samples, a total of 723 samples in cross-sectional studies and 153 cases and 97 controls in case-control studies were included, out of which 199/538 (36.98%) and 155/247 (62.75%) samples were positive for anti-*T. gondii* IgG and IgA antibodies in cross-sectional studies and 86/128 (67.18%), 1/53 (1.88%), and 78/153 (50.98%) cases and 4/47 (8.51%), 0/47 (0%), and 12/97 (12.37%) controls were positive for anti-*T. gondii* IgG, IgM, and IgA antibodies in case-control studies, respectively. The results suggested that antibody levels in saliva and tear samples in humans could be useful for the diagnosis of toxoplasmosis, especially ocular toxoplasmosis using tear samples.

Keywords: *Toxoplasma gondii*, non-invasive samples, saliva, tear

Introduction

Toxoplasma gondii (*T. gondii*) is widely distributed around the world, and felids are its definitive hosts. Moreover, a wide range of warm-blooded animals, including humans and ruminants, act as intermediate hosts [1,2]. Sexual reproduction occurs only in definitive hosts, since the enzyme delta-6-desaturase, which is needed for linoleic acid metabolism is inactive only in the intestines of these mammals leading to a systemic increase in linoleic acid [3]. Human infection occurs often orally or via blood transfusion from infected donors, organ transplantation, and vertical transmission during pregnancy from mother to fetus [4]. The infection is generally asymptomatic or leads to a mild, self-limiting disease in immunocompromised adults. Nevertheless, in immunocompromised patients (AIDS patients, individuals with malignancies receiving chemotherapy, and organ transplant recipients) and in fetuses with congenital toxoplasmosis, *T. gondii* often has a range of serious and life-threatening clinical symptoms [5,6]. The prevalence of *T. gondii* infection varies in different parts of the world and this difference in prevalence depends on climatic and geographic factors, hygiene standards, feeding and cultural habits, and profession [7–9].

The diagnosis of *T. gondii* infection in intermediate hosts is usually based on serology, histopathology, PCR, immunohistochemistry, and bioassays in mice [10–12]. Today, the diagnosis of human toxoplasmosis is mainly based on detecting of anti-*Toxoplasma* antibodies in serum samples using various serological methods, although serum collection is an invasive method. Therefore, the use of other biological fluids, such as saliva and tear, will be more useful for *Toxoplasma* screening,

particularly in field conditions. These sampling methods are less hazardous, non-invasive, more acceptable for the patient, and easier and cheaper than blood sampling [13]. Therefore, this study provides a summary of available data on the use of non-invasive specimens (saliva and tear) in the diagnosis of toxoplasmosis.

Methods

Study design and protocol registration

This systematic review was conducted in accordance with the items reported in the PRISMA statement (www.prisma-statement.org). This protocol was registered on the website of the International Prospective Register of Systematic Reviews with the identifier Central Registration Depository of 42020153541.

Literature search, study selection, and data extraction

This study performed computer-aided searches of the following electronic databases: PubMed, Web of Science, ScienceDirect, ProQuest, and Scopus up to the 20th of August 2021. The following keywords and their combinations were used: "*Toxoplasma gondii*", "*T. gondii*", "toxoplasmosis", "non-invasive samples", "saliva", "tear", "diagnosis", "detection", "serological", and "molecular". References to all retrieved articles were hand-searched for additional references. Articles returned from the electronic database searches were imported into Endnote (version X8), and the titles and abstracts of all papers were examined by the two authors. The contradictions were resolved by discussion and consensus with a third reviewer. Then, the data were transferred to Microsoft Excel 2016 datasheets. The data extracted from every study included first author, publication year, country, host, type of sample, sample size, antibody type, and results of saliva, tear, and serum as well as sensitivity and specificity of tests.

Inclusion and exclusion criteria

The inclusion criteria were cross-sectional studies conducted on saliva and tear as non-invasive samples by serological and molecular methods. Moreover, original research articles published in the English language with available full text were included in this study. Other inclusion criteria were articles that used saliva and tear as diagnostic tools for toxoplasmosis. On the other hand, studies on other non-invasive specimens such as milk and urine as well as thesis and conference papers were excluded from the study.

Results

Characteristics of study and search results

Overall, 910 articles published from 1994 to 2021 (27 years) worldwide were identified in the literature search. After removing duplicates and non-eligible papers based on title and abstract, 23 articles (16 saliva and 7 tears) were entered into the systematic review. Figure 1 briefly shows the search process in this systematic review article.

Results of studies on saliva samples

The included studies in this systematic review study were conducted in Brazil (n=4), followed by the USA (n=3), England, Switzerland, and France (n=2), India, Tunisia, and Mexico (n=1). Fifteen studies had a cross-sectional design and one study was a case-control [14]. In the study of Singh et al. [14], the prevalence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies of *T. gondii* in serum and saliva samples of 100 cases and 25 controls were investigated out of which 25 and 16 serum samples were positive for anti-*T. gondii* IgG and IgM antibodies. Also, in saliva samples, 20 (20%) and 25 (25%) cases and 0 (0%) and 1 (4%) controls were positive for anti-*T. gondii* IgG and IgM antibodies, respectively. Dave et al. [6], in their study, examined the prevalence of *T. gondii* in serum and saliva, they did not report the number and percentage of positive cases. In 15 cross-sectional studies, a total of 1315 serum samples were examined for the seroprevalence rate of anti-*T. gondii* antibodies, out of which 337/1205 (27.97%), 64/511 (12.52%), and 35/170 (20.59%) samples were positive for anti-*T. gondii* IgG, IgM, and immunoglobulin A (IgA) antibodies, respectively. In addition, a total of 4338 saliva samples were examined for the prevalence of *T. gondii* infection, out of which 718/3848 (18.66%), 30/200 (15%), and 60/122 (49.18%) samples were positive for anti-*T. gondii* IgG, IgM, and IgA antibodies, respectively. There was an experimental section in the study of Campero et al. that the oral fluids were collected from eight sows experimentally inoculated and from three negative control sows at different time points after inoculation. Sows experimentally inoculated with *T. gondii* showed positive immunoblot results for IgA (8/8 sows) and IgG (7/8

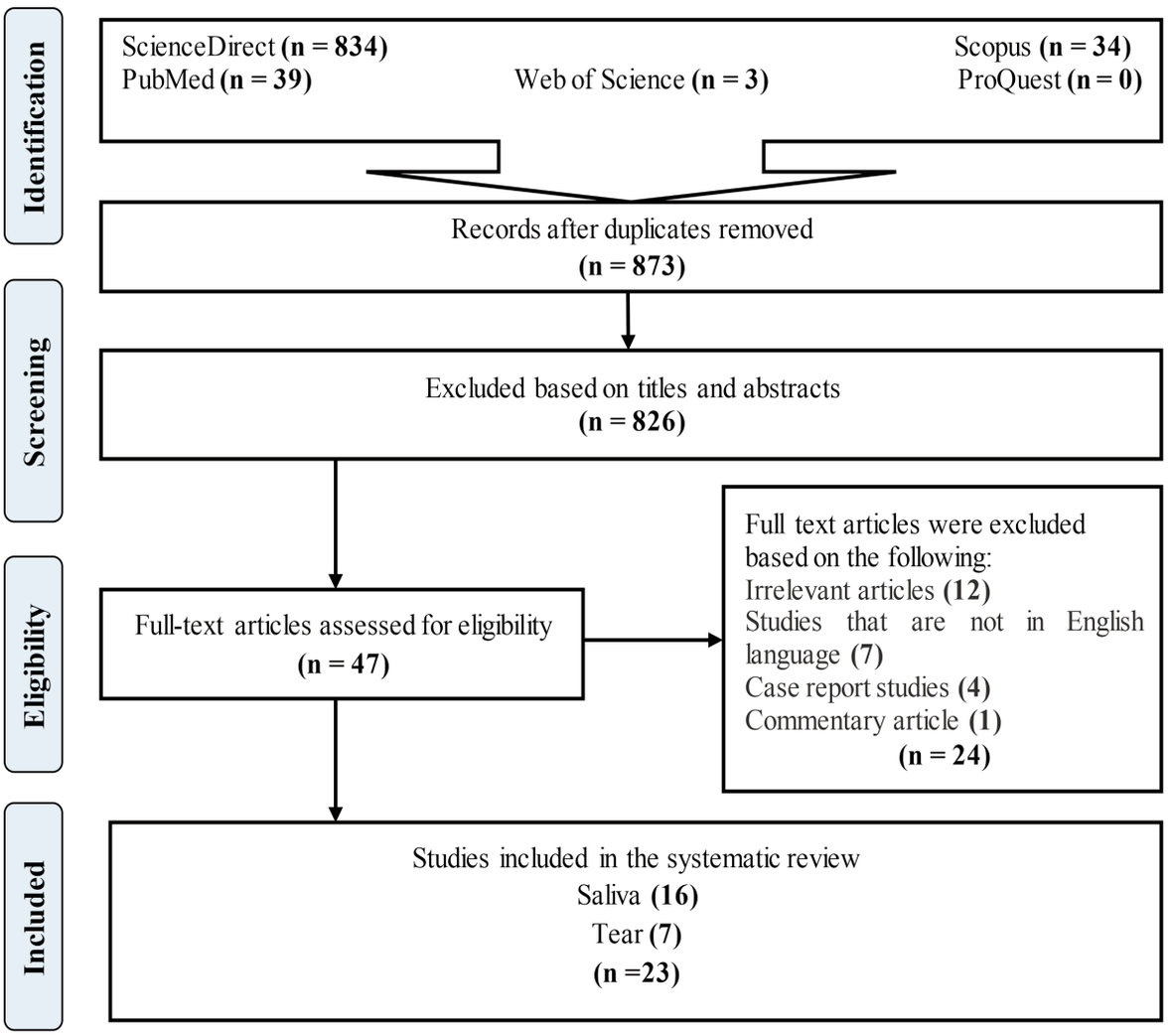

Figure 1. Flow diagram of the study design process

sows) against TgSAG1 antigen in oral fluid at 1.5 weeks post-inoculation [15], but the frequency of detection of both Ig isotypes decreased over time, with intermittent positive immunoblot results [16].

Results of studies on tear samples

The prevalence of *T. gondii* in tear samples was investigated in seven articles (3 cross-sectional and 4 case-control studies). As illustrated in table 2, the identified studies were conducted in the three countries of the world, including Brazil (n=4), Netherlands (n=2), and Egypt (n=1). Six hundred and sixty-nine samples of serum in four cross-sectional studies were evaluated for anti-*T. gondii* antibodies using various serological techniques such as enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), Sabin-Feldman dye test, and western blot out of which 367/640 (57.34%), 56/640 (8.75%), and 16/62 (25.80%) samples were positive for anti-*T. gondii* IgG, IgM, and IgA antibodies, respectively. In the study of Lynch et al. [17], the number of positive serum samples for IgG and IgM antibodies was not clearly stated. Also, in these four studies, the IgA antibody was evaluated only in the study of Meek et al. [18]. In three case-control studies, 220 cases and 97 controls were evaluated for anti-*T. gondii* antibodies in serum samples using serologic tests out of which 142 /220 (64.55%), 8/220 (3.63%), and 47/220 (21.36%) cases and 74/93 (79.57%), 0/97 (0%), and 26/97 (26.80%) controls were positive for anti-*T. gondii* IgG, IgM, and IgA antibodies, respectively. In the study of Ronday et al. [19], IgG levels were examined in only 23 of 27 individuals in the control group. Therefore, a total of 723 tear samples in four cross-sectional studies and 153 cases and 97 controls in case-control studies were included in this systematic review out of which 199/538 (36.98%) and 155/247 (62.75%) tear samples were positive for anti-*T. gondii* IgG and

Table 1. Baseline of descriptive studies on saliva and serum samples included in this systematic review

No	First author (year)	Country	Host(s)	Sample(s)	Methods	Detected antibody	Sample size (n)	Serological results of saliva n (%)	Serological results of serum n (%)	Sensitivity and sensitivity of tests	Comparison results
1	Hajeer et al. (1994) [38]	England	Human (three groups: recently infected patients, chronically infected, and seronegative adults)	Saliva and serum	DT, IgM-ELISA, DA, ISAGA, and western blotting	IgG, IgM, IgA	52 (recently infected patients: 27, chronically infected: 8, seronegative adults: 17)	Recently infected patients: IgG: 13 (48.14) and IgM: 7 (25.92), chronically infected: (IgG: 0 and IgM: 0), and seronegative adults: (IgG: 0 and IgM: 0)	Recently infected patients: (IgG: 27 and IgM: 22), chronically infected: (IgG: 0 and IgM: 0), and seronegative adults: (IgG: 0 and IgM: 0)	–	All serum IgA blots from the recently infected patients gave a positive reaction. In the case of chronically infected adults, serum IgA blots showed weak reactions. None of the seronegative adults gave positive reactions with serum IgA blots. IgA was detected by western blotting in the saliva of people tested from all three groups. Serum titer increased rapidly, peaked at 34 days post infection (p.i.) and became negative after 83 days p.i. Serum DT titres peaked at 83 days p.i. and stayed high until the last sample collected, 146 days p.i. Salivary IgM was detected 34 days post infection and was negative by 81 days p.i. Salivary IgG peaked at 74 days p.i., dropped quickly and was negative by 81 days p.i., which coincided with that of salivary IgM. Salivary IgG detected the 30 and 38 kDa proteins, while IgA detected mainly the 35 kDa protein. Both salivary IgG and IgA were negative by immunoblotting by 112 days p.i.
2	Dave et al. (1997) [6]	England	Human	Saliva and serum	DA and ELISA	IgG and IgM	539	–	–	The highest sensitivity was obtained with the DA test on direct saliva (49.2%). The lowest sensitivity was with the ELISA IgM test on direct saliva (7.2%). The specificities for all of the tests were over 95%.The highest and lowest positive predictive values were with ELISA IgG on direct saliva (99.00%)) and IgM ELISA on direct saliva (57.1%)	The results show that saliva has only a limited use in the detection of acute or latent *Toxoplasma* infections

Table 1. Baseline of descriptive studies on saliva and serum samples included in this systematic review

3	Loyola et al. (1997) [21]	Brazil	Human	Saliva and serum	ELISA	IgG and IgA	60	IgG: 43 (71.7) and IgA: 18 (30)	IgG: 12 (20) and IgA: 12 (20)	–	No association was observed when the *Toxoplasma*-specific IgG reactive and non-reactive serum samples were compared with the reactive and non-reactive saliva samples for this class of immunoglobulin. On the other hand, a significant association was observed when the *Toxoplasma*-specific IgA reactive and non-reactive serum samples were compared with the reactive and non-reactive saliva for this type of antibody. Results show that the detection of salivary IgA reflects the serum level of this isotype but salivary IgG does not.
4	Singh et al. (2005) [14]	India	Human (HIV positive)	Saliva and serum	ELISA	IgG and IgM	100 patients and 25 controls	Patients: IgG: 20 (20) and IgM: 25 (25) and controls: IgG: 0 (0) and IgM: 1 (4)	Patients: IgG: 25 (25) and IgM: 16 (16)	Sensitivity: IgG: 64% and IgM: 81.25% and Specify: IgG: 94.67% and IgM: 85.71%.	Saliva samples can be used as an alternative to serum samples to detect anti-*Toxoplasma* antibodies, particularly IgM, for the diagnosis of *Toxoplasma* encephalitis in HIV positive.
5	Stroehle et al. (2005) [39]	Switzerland	Human	Saliva and serum	ELISA, Vidas, and immuno blotting	IgG	201	58 (28.86)	IgG: 59 (29.4) and IgM: 0/59 (0)	The saliva immunoblot test exhibits a specificity of 100% and a sensitivity of 98.5%.	The sera of 59 individuals showed IgG antibodies against *T. gondii* by ELISA, Vidas, and immunoblotting; 58 of these were also positive for anti-*T. gondii* IgG in the saliva immunoblot.
6	Griffin et al. (2011) [40]	USA	Human	Saliva and serum	ELISA and luminex multiplex immunoassay	IgG and IgA	10	2 (10)	2 (10)	–	Results of Luminex tests for salivary IgG antibodies to *T. gondii* matched those of diagnostic serological ELISA tests. IgG seropositive individuals based on the ELISA also had the strongest response of salivary antibodies.
7	Sampaio et al. (2013) [41]	Brazil	Human students	Saliva and serum	Dot-ELISA and protein A IgG capture ELISA assay	IgG	100	19 (19)	–	–	Dot-ELISA results demonstrated consistency between the serum and saliva samples.

Table 1. Baseline of descriptive studies on saliva and serum samples included in this systematic review

No	First author (year)	Country	Host(s)	Sample(s)	Methods	Detected antibody	Sample size (n)	Serological results of saliva n (%)	Serological results of serum n (%)	Sensitivity and sensitivity of tests	Comparison results
8	Bel-Ochi et al. (2013) [42]	Tunisia	Human patients immune and non immune	Saliva and serum	ELISA	IgG	91	OD= 0.14: 54 (59.34) and OD=0.29: 33 (36.26)	OD= 0.14: 49 (53.85) and OD=0.29: 33 (36.26)	The rSAG1 serum-based ELISA detected specific IgG with 100% sensitivity and specificity. The positive percent agreement and negative percent agreement (NPA) between the serum-based and saliva-based tests varied according to the selected optical density threshold in saliva. Thus, for a selected cutoff of 0.14, the PPA was 100% and the NPA was 88.1%, whereas for a selected cutoff of 0.29, the PPA was 67.3% and the NPA was 100%.	For an OD threshold of 0.14, the overall percent agreement between saliva-based and serum-based tests was 94.5%, the PPA was 100%, and the NPA was 88.1%, whereas for an OD threshold of 0.29, the overall percent agreement between the 2 tests was 82.4%, the PPA was 67.3%, and the NPA was 100%. If the rSAG1 serum-based ELISA is assumed to be a gold standard and the rSAG1 saliva-based ELISA is being measured against it, at a specificity of 100%, the sensitivity of the saliva-based test was 69.4%, and at a sensitivity of 100%, the specificity of the test was 88.1%.
9	Chapey et al. (2015) [43]	France	Human	Oral fluid and serum	ELISA	IgG, IgM, and IgA	322 [pilot study: 212 and target population: 110 (cases: 93 and controls: 17)]	Pilot study: the levels of anti-*T. gondii* IgG in oral fluid were significantly higher in seropositive than in seronegative patients and target population: cases: 22 (23.65) and controls: 9 (52.94) IgG positive	Pilot study: 195 (91.98) IgG positive and target population: 23 [cases: 23 (24.73) and controls: 0 (0) IgA and IgM positive]	The sensitivity and specificity of the test were 67.9% and 80.3%, respectively, and the probability of not having a congenital infection when the test on oral fluid was negative was 99%.	These data demonstrate a good parallel in the evolution of IgG titers in oral fluid and sera.
10	Canedo-Solares et al. (2017) [44]	Mexico	Human (woman)	Saliva	Indirect ELISA	IgG, IgA, IgG1, IgG2, IgG3, and IgG4	91	–	IgG: 50 (54.9) and low avidity: 4 (4.4)	IgG1 antibodies had a sensitivity of 0.67 and a specificity of 0.71 in serum and sensitivity of 0.40 and a specificity of 0.85 in saliva. No other antibody gave values of sensitivity and specificity higher than 0.50 in saliva.	Findings indicated no correlation between antibodies of serum and saliva. For IgG and IgG1, several individuals had antibodies in both samples, and few of them were positive only in saliva or serum. Regarding IgG2, IgG3, IgG4 and IgA, most cases were positive in saliva but negative (or at very low level) in serum or vice versa, with some exceptions for IgG3 and IgG4.
11	Augustine et al. (2017) [20]	USA	Human	Saliva	Multiplex immunoassay	IgG	2078	166 (8)	–	–	–

Table 1. Baseline of descriptive studies on saliva and serum samples included in this systematic review9

12	Macre et al. (2019) [45]	Brazil	Human	Saliva	ELISA	IgG	164 and 45	82 (50) and 24 (53.33)	–	–	–
13	Sampaio et al. (2019) [46]	Brazil	Human	Saliva	ELISA	IgG	249	21 (8.4)	–	Specificity of the saliva assays were greater than 95%.	–
14	Li et al. (2019) [47]	France	Human	Saliva and serum	Architect and AxSYM Abbott IgG and IgM assays, DT, and multiplexed serology assay using novel plasmonic gold chips	IgG and IgM	148	Levels of IgG tested on plasmonic gold chips: 74 (50) and IgM: 23 (15.54)	[Abbott IgG test: 78 (52.70) and Abbott IgM: 17 (11.48)] and [levels of IgG tested on plasmonic gold chips: 78 (52.70) and IgM: 19 (12.83)]	In serum and whole blood, sensitivity and specificity of multiplex *T. gondi* IgG was 100% in pGOLD when compared to commercial test results in serum. In saliva, sensitivity and specificity for *T. gondii* IgG was 100% and to detect *T. gondii* IgM in saliva was sensitivity and specificity of 100% and 95.4%, when compared to commercial test results in serum.	The correlation coefficient between saliva and serum detection on pGOLD for *T. gondii* IgG and IgM was 0.88 and 0.81 respectively that highlighted an almost perfect agreement between serum and saliva test results on the pGOLD platform.
15	Campero et al. (2020) [16]	Switzerland	Pig	Oral fluid and serum	ELISA and immunoblot	IgG and IgA	552 (n = 42 groups with three to 29 pigs, average 13 animals/group)	IgG: 22/62 (35.5) and IgA: 42/62 (67.7)	60 (10.9)	–	Sixty out of 552 (10.9%) individually tested fattening pigs yielded positive results in the serum-ELISA. The seropositive animals belonged to 13 of the 42 groups. Eleven groups had 3.4 to 13% seropositive pigs and two groups had 91 and 92% seropositive pigs. The remaining 29 groups contained 100% seronegative animals. Analysis of pooled oral fluid from fattening pigs yielded positive results for both IgA and IgG (with a stronger signal for IgA) only in the two groups with the highest rates of seropositive pigs (91 and 92%). All 11 groups with up to 13% seropositive pigs and the 29 groups with 100% seronegative pigs yielded negative oral fluid-immunoblot results for both Ig isotypes.
16	Augustine et al. (2021) [48]	USA	Human	Saliva	Multiplex immunoassay	IgG	478	109 (22.8)	—		—

Explanations: DT: dye test, ELISA: enzyme-linked immunosorbent assay, DA: direct agglutination, ISAGA: immunosorbent agglutination assay, IgG: immunoglobulin G, IgM: immunoglobulin M, and IgA: immunoglobulin A

Table 2. Baseline of descriptive studies on tear and serum samples included in this systematic review

No	First author (year)	Country	Host (s)	Sample (s)	Method(s)	Detected antibody	Sample size (n)	Results of tear n (%)	Results of serum n (%)	Sensitivity and sensitivity of tests	Comparison results
1	Ronday et al. (1999) [19]	Netherlands	Human	Tear and serum	ELISA, IFA, and PCR	IgA, IgG, and IgM	155 (78 patients with presumed ocular t oxoplasmosis and 77 patients with uveitis that was not clinically suspected to be ocular toxoplasmosis) and 27 control subjects	Patients [IgG: 57/88 (65), IgM: 1/13 (7.69), IgA: 46/88 (52) and PCR: 12/44 (27.27)] and controls [IgG: 0/27 (0), IgM: 0/27 (0), and IgA: 0/27 (0)]	Patients [IgG: 88 (56.77), IgM: 8/88 (9), and IgA: 14/88 (16)] and controls [IgG: 17/23 (73.91), IgM: 0/27 (0), and IgA: 0/27 (0)]	The sensitivity of PCR testing was 27%, whereas the sensitivity of determining intraocular anti-*T. gondii* IgG and IgA production was 73% and 55%, respectively. The conventional tests (intraocular anti-*T. gondii* IgG antibody production and DNA testing) had a comb ined sensitivity of 77%. Additional detection of intraocular anti-*T. gondii* IgA increased sensitivity by 14%, to a total of 91% and the specificity of all tests was 100%.	Detection of anti–*T. gondii* IgA antibodies in serum is used to establish recently acquired infection. The presence of intraocular anti-*T. gondii* IgA antibodies not only during postnatally acquired but also during recurrent disease could be the result of the unique properties of the local environment of the antibody-producing plasma cells.
2	Meek et al. (2000) [18]	Netherlands	Human	Tear and serum	DT and western blot	IgA and IgG	62	IgG: 16 (25.80) and IgA: 51 (81)	SF dye test [IgG: 14 (23)] and western blot: [IgG: 46 (74.19), IgM: 29 (46.77), and IgA: 16 (25.80)]	–	There was no apparent relation between chronic infection and presence of anti-*T. gondii* IgA in tears. The avidity of the anti-*T. gondii* IgA antibodies in tears was similar to the avidity of serum IgG antibodies.
3	Lynch et al. (2004) [49]	Brazil	Human	Tear and serum	ELISA and IFA	IgA and IgG	75 (25 patients and 50 healthy controls)	Patients [IgA: 21 (84)] and healthy controls [IgA: 11 (22)]	Patients [IgG: 25 (100), IgM: 0 (0) and IgA: 25 (100)] and healthy controls [IgG: 50 (100) and IgA: 25 (50)]	84% sensitivity and 78% specificity	Considering the strong association found between the disease and the lacrimal sIgA production as well as the mild conditions for obtaining tear samples, the proposed method for determining anti-*T. gondii* sIgA should be helpful in the APUPT diagnosis.

Table 2. Baseline of descriptive studies on tear and serum samples included in this systematic review

4	Ismail et al. (2007) [26]	Egypt	Human	Tear and serum	ELISA	IgA, IgG, IgM, and IgG avidity	Group (I): 14 patients with uveitis, Group (II): 26 patients without active uveitis, group (III): 20 healthy individuals	Group (I): [IgG: 10/14 (71), IgM: 0 (0), and IgA: 9/14 (64), and IgG avidity: low: 9/14 (64) and high: 1/14 (7)], Group (II): [IgG: 19/26 (73), IgM: 0 (0), and IgA: 2/26 (8), and IgG avidity: low: 0/26 (0) and high: 19/26 (73)], and group (III): [IgG: 4/20 (20), IgM: 0 (0), and IgA: 1/20 (5), and IgG avidity: low: 0/20 (0) and high: 4/20 (20)]	Group (I): [IgG: 8/14 (57), IgM: 0 (0), and IgA: 5/14 (36), and IgG avidity: low: 2/14 (14) and high: 6/14 (43)], group (II): [IgG: 21/26 (81), IgM: 0 (0), IgA: 3/26 (12), and IgG avidity: low: 0/26 (0) and high: 21/26 (81)], and group (III): [IgG: 7/20 (35), IgM: 0 (0), IgA: 1/20 (5), and IgG avidity: low: 0/20 (0) and high: 7/20 (35)]	–	The tear fluid can be considered as a promising candidate for detection of local ocular antibodies because its collection is simple, easy and noninvasive.
5	Lynch et al. (2009) [32]	Brazil	Human	Tear and serum	ELISA and IFA	IgA, IgG, and IgM	156 (APUPT: 82 and APUOE: 74)	APUPT [IgA: 54/82 (65.85)] and APUOE [IgA: 21/74 (28.37)]	APUPT [IgG: 50/52 (96.2) and IgM: 1/52 (1.9)] and APUOE [IgG: 34/50 (68.00) and IgM: 0/50 (0)]	65.9% sensitivity and 71.6% specificity	sIgA reactivity was higher in the tears of patients with active posterior uveitis due to *T. gondii*.
6	Lynch et al. (2011) [17]	Brazil	Human	Tear	ELISA	IgA	29	Active phase: 29 (100) and inactive phase: 7 (24.13)	–	–	–
7	Mangiavacchi et al. (2016) [50]	Brazil	Human	Tear and serum	ELISA	IgA, IgG, and IgM	476	[ROC curves for anti-TgERP IgG: 183 (48·1) and S.D. cut-off for anti-TgERP IgG: 120 (31·6)]	Conventional ELISA: [IgG only: 225 (47.27), IgM only: 15 (3.1), IgG and IgM: 12 (2.5) and IgG and/or IgM: 249/476 (52.3)] and [ROC curves for anti-TgERP IgG: 190/380 (50) and S.D. cut-off for anti-TgERP IgG: 73/380 (19.2)]	–	The recommendation of using salivary IgA to investigate the prevalence of toxoplasmosis, with regard to its advantage over serum as a medium for antibody detection due to its non-invasive nature.

Explanations: DT: dye test, ELISA: enzyme-linked immunosorbent assay, IFA: indirect fluorescent antibody test, PCR: polymerase chain reaction, IgG: immunoglobulin G, IgM: immunoglobulin M, and IgA: immunoglobulin A

IgA antibodies in cross-sectional studies and 86/128 (67.18%), 1/53 (1.88%), and 78/153 (50.98%) cases and 4/47 (8.51%), 0/47 (0%), and 12/97 (12.37%) controls were positive for anti-*T. gondii* IgG, IgM, and IgA antibodies in case-control studies, respectively.

Discussion

This systematic review shows that saliva and tear samples can be used instead of serum to diagnose toxoplasmosis in humans and animals around the world. Saliva can be useful in diagnosing many infections. According to table 1, the lowest and highest prevalence rates of anti-*Toxoplasma* IgG antibodies in saliva samples were related to the studies performed by Augustine et al. (166/2078: 80%) [20] and Loyola et al. (43/60: 71.7%) [21]. Saliva can be a very important source of antibodies for certain groups of patients, such as children. Vyse et al. [22] reported that saliva sampling is more acceptable in children compared to serological techniques involving needle puncture. Avoiding invasive needles increases the confidence and perseverance of the treated person and helps prevent absenteeism. In addition, the collection of saliva is more cost-effective and safe than invasive blood collection procedures [22,23]. Using saliva samples to diagnose toxoplasmosis in a country like France can be important. In France, newborns born to mothers who developed toxoplasmosis during pregnancy are fully screened at birth, including serological tests for specific IgG, IgM, and IgA. Maternal IgG crosses the placenta and its presence in neonatal serum cannot be considered a sign of congenital infection. To rule out a congenital *T. gondii* infection, a specific IgG test must be negative in the first year of life, indicating that the infant has not secreted IgG and has removed the maternal antibodies completely [24]. In oral fluid, the three main antibodies (IgA secreted by the salivary glands and IgG and IgM, which are serum secretions of capillaries along the gums), as well as most blood components, can be detected at lower concentrations [25]. Meanwhile, some studies such as Dave et al. [6] reported that saliva is not a good tool for screening for *Toxoplasma* in pregnancy. Poor sensitivity to saliva testing may be due to undetectable levels of IgG and IgM in the saliva of patients whose serum was positive for IgG and IgM, or because tests failed to detect the IgG and IgM that are actually present in saliva. The low sensitivities for *Toxoplasma* testing may be due to improper use of the device by patients or the long interval between the use of saliva collection devices and their receipt by the laboratory [6].

Table 2 showed that the lowest and highest prevalence rates of anti-*Toxoplasma* IgA antibodies in tear samples of patients using serology methods were observed in studies conducted by Ismail et al. (11/40: 27.5%) [26] and phase active of Lynch et al. (29/29: 100%) [17]. Posterior uveitis is related to *T. gondii* in 30–50% of cases [27,28]. Uveitis is a major cause of blindness worldwide and toxoplasmic retinochoroiditis is the most common form of posterior uveitis [29]. In the eye, depending on the technique used to measure antibodies, the local production of antibodies in the aqueous humour, compared to the level of serum antibodies, can be determined in the sensitivity range of 41–80% and with a specificity of ~90% [30–32]. The intraocular compartment contains relatively high levels of transforming growth factor β, a cytokine that is an important factor in directing B cells to produce IgA antibodies, which may explain the production of anti-*T. gondii* IgA antibody during ocular toxoplasmosis [33,34]. The production of anti-*T. gondii* IgA antibodies in the eye may prevent the activation of the complement system and limit the blindness caused by inflammation [19]. To investigate the association between of anti-*Toxoplasma* secretory IgA antibodies and the diagnosis of ocular toxoplasmosis, it is necessary to determine IgA levels at different stages of the disease. The anti-*T. gondii* IgA plasma levels increase in the acute phase of systemic infection and remain high for 9 months and then decrease to normal levels [35]. Stimulus from ocular cysts or other tissues can lead to the chronic presentation of *Toxoplasma* antigens by asymptomatic degradation of these cysts, which ultimately leads to the maintenance of positive secretory IgA levels in the mucosa by circulating lymphocytes [29,36]. In places with a high prevalence of *Toxoplasma*, chronic stimulation of the intestinal mucosa may occur due to constant contact with the parasite. The frequent uptake of tissue cysts or oocysts may stimulate memory B-lymphocytes in the intestinal mucosa and produce secretory IgA. When these active lymphocytes enter the bloodstream through the lymphatic system, lymphocytes can stimulate other mucosal surfaces and produce specific antibodies [36,37].

This systematic review has several limitations as

follows: 1) a limited number of studies have examined the saliva and tear as a non-invasive and alternative sample for serum in the diagnosis of toxoplasmosis, 2) different diagnostic methods with various specificities and sensitivities were used to measure the levels of *T. gondii* antibodies, and 3) the use of English language articles due to lack of resources for translation. As far as we know, this is the first systematic review of the detection of *T. gondii* antibodies in tear and saliva samples. The data obtained in this study show that tears and saliva can be a good alternative to serum in the diagnosis and screening of toxoplasmosis in humans and animals. Given that there are still few studies on the role of tear and saliva in the diagnosis of toxoplasmosis, the research team hopes that providing accurate information on the prevalence of toxoplasmosis in tear and saliva samples, can encourage more extensive research to help diagnose, control, and treatment this parasite in Iran and around the world.

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