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Review Article

Antigen-Based Diagnosis of Human Giardiasis: A Systematic Review and Meta-Analysis

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Abstract

Background: We aimed to present a systematic review and meta-analysis of studies that used antigen-based assays for the diagnosis of human giardiasis.

Methods: All the related published literature cited within PubMed, ISI web of science, Google Scholar, Embase, and Scopus, were searched up to December 2021. The search terms, both as MeSH terms and text words, were "*Giardia*", "*Giardia lamblia*", "*Giardia intestinalis*", "giardiasis", combined with "diagnosis", "antigen detection", serodiagnosis, or serological diagnosis. The required data was extracted from the papers. Pooled estimates of sensitivity and specificity were obtained and forest plots and summary receiver operating characteristics (SROC) plots were used to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results: The search of databases found 1683 papers, of which 46 articles fulfilled our eligibility criteria. The sensitivity of antigen-based methods for the diagnosis of human giardiasis ranged from 45% (95% CI: 31-59%) to 100% (95% CI: 100-100%) and the pooled estimate of sensitivity was 92% (95% CI: 90-93%). The pooled estimated specificity was 97% (95% CI: 96-98%), ranged from 81% (95% CI: 68-89%) to 100% (95% CI: 98-100%). The summary estimate of PPV and NPV were 92 % (95% CI: 90-93%) and 97% (95% CI: 96-98%) respectively. Comparing the performance of the antigen detection assays by region revealed a significant difference in the assay's performances in different regions of the world.

Conclusion: The antigen-based detection methods have acceptable and satisfactory performance in the diagnosis of human giardiasis. The task ahead is to identify more specific target antigens and design simpler, cheaper, and more sensitive methods for the diagnosis of this common worldwide-distributed parasitic infection.

Introduction

Giardia lamblia is a non-invasive protozoan parasite that annually infects more than 280 million people, especially children, worldwide (1). Although giardiasis is recognized as an endemic parasitic infection throughout the world, most cases are reported from tropical countries with poor hygiene. The rate of infection in developed and developing countries is reported to be 1–7% and 4 – 43% respectively (2, 3). Giardiasis is an important parasitic infection especially in children where its association with growth restriction has been well documented. Proper and appropriate diagnosis of giardiasis is necessary for timely and effective treatment.

Diagnosis of giardiasis

Several different diagnostic systems have been described for the diagnosis of giardiasis: among them are microscopic diagnosis, molecular methods, and immunoassays such as ELISA for the detection of *Giardia* antigen or anti-*Giardia* antibodies, and Direct Fluorescence Antibody (DFA) test, which detects antigens present on the cell wall of *Giardia* cysts (4-7).

Microscopy

The traditional method for the diagnosis of giardiasis is based on the detection of cysts or trophozoites of *Giardia* through microscopy examination of fecal samples. Although the microscopic diagnosis of giardiasis is laborious, it is still considered the gold standard for the diagnosis of giardiasis. However, as cyst or trophozoite excretion in giardiasis is intermittent, using this method may cause a false negative outcome. Besides, the microscopy depends on the experience of the microscopist and the number of examined fecal samples. Usually, examination of one fecal sample results in the diagnosis of 60 to 80% of cases, two fecal samples examination (preferably taken two or three days apart) will allow the detection of 80 to 90%, and three stool samples will allow the diagnosis of over 90% of cases (8).

Antigen detection

Antigen detection methods rely on the detection of *Giardia* antigens in human fecal samples. One of the antigens that have been considered in the diagnosis of *Giardia* is the 65 kDa (GSA65) antigen. This antigen is present in both trophozoites and cysts of the parasite. The use of this antigen in the ELISA system detects 30% more cases than the microscopic method. The sensitivity and specificity of this antigen in the ELISA system have been reported to be 95% and 100%, respectively (9).

One of the advantages of antigen-based detection methods over microscopy is the possibility to analyze a single stool sample in fresh/preserved or frozen specimens, while the microscopy requires, inevitably, the analysis of at least three fresh stool samples (10). Instead, the microscopy benefits from the low cost and the possibility of observation of other microorganisms in the same sample (11). Several immunoassays have been developed for simultaneous detection of *Giardia*, *Cryptosporidium*, and *Entamoeba* species antigens in human fecal samples. The commercial Tri-Combo parasite screen test (TechLab, Inc., Blacksburg, VA) simultaneously detects *Giardia* sp., *Entamoeba histolytica* and *Cryptosporidium parvum*. Although this test is not able to distinguish these three parasites, it is nevertheless a good way to screen samples suspected of having intestinal protozoa. Acceptable sensitivity (91-97%) and specificity (97-99%) have been reported for this test in different studies (12, 13).

The differences in the performance of serological antigen-based detection methods in the diagnosis of giardiasis have been attributed to various factors, including the low number of parasites in the sample, intermittent shedding

of cysts and *Giardia* antigens, cross-reactivity with other protozoa or parasites antigens, and the intervening role of stool preservative solutions (10, 14, 15). The advantages of antigen-based methods in the diagnosis of giardiasis include the ability of these methods to diagnose the disease before the presence of cysts or trophozoites in the patient's stool sample, the speed of these methods; it does not rely on professional staff, and the ability to screen a large number of samples. Moreover, the ability of these methods to monitor the outcome of treatment can be added to their benefits.

Antibody detection

The main challenge with antibody-based detection methods for the diagnosis of giardiasis is that the antibody remains for a relatively long time after spontaneous recovery or drug treatment (4, 16-18). Therefore, the presence and detection of antibodies in the human serum do not indicate an active *Giardia* infection.

In recent years, various serological approaches have been developed for the diagnosis of human giardiasis, which needs to be evaluated, summarized, and analyzed. Here, we present a systematic review and meta-analysis of studies that have been using antigen-based assays for the diagnosis of human giardiasis.

Methods

The current systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). All the related published literature cited within PubMed, ISI web of science, Google Scholar, Embase, and Scopus, (in English and in Persian) were searched up to December 2021. The search terms, both as MeSH terms and text words, were "*Giardia*", "*Giardia lamblia*", "*Giardia intestinalis*", "giardiasis", combined with "diagnosis", "antigen detection", serodiagnosis, or serological diagnosis. The considered inclusion criteria were articles that evaluated the antigen detection method for the diagnosis of human giardiasis, and the availability of the absolute numbers of true positive, true negative, false positive, and false negative in the presented data. The exclusion criteria were: 1) studies reporting other diagnostic methods (i. e., molecular detection); 2) those articles on *Giardia* diagnosis in the veterinary field; 3) Studies in which the control group was not properly identified; 4) studies other than original articles (i. e., review, case reports, and case series); 5) studies in which the number of samples studied was insufficient (less than 10 positive samples).

Quality assessments of the articles that met inclusion criteria were done using Quada-2 (quality assessment of diagnostic accuracy studies) tools. The following information was extracted from each article. 1) sample size (number of giardiasis patients, number of negative/cross controls cases, 2) the reported sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), 3) country where the samples originated 4) type of diagnostic assay, 5) number of true/false positive and true/ false-negative cases. Assays based on both homemade antigens or commercial kits were included in the review.

Statistical analysis

We used R 3.2.2 with Meta-Analysis of Diagnostic Accuracy (MADA) as well as Hierarchical Summary Receiver Operating Curve (HSROC) packages. Pooled estimates of sensitivity and specificity were obtained and forest plots and summary receiver operating characteristics (SROC) plots were used to calculate sensitivity, specificity, PPV, NPV, OR, and to provide a ROC curve.

Results

Through database searching, 1683 articles were found on the initial search, of which 461 titles were overlapped. The title and abstracts of the remaining 1222 articles were screened

and 1134 articles were deemed ineligible and excluded. Following reading the full text of the remaining 88 articles, 42 articles were excluded, as they did not meet the inclusion criteria. Thus, 46 articles met the eligibility criteria, which constituted the basis of this meta-analysis (Fig. 1).

All of the included studies evaluated the antigen detection assays (by different available methods) for the diagnosis of human giardiasis where the microscopic method was used as the main reference test. Table 1 summarizes the characteristics of the included studies. Since in some studies several methods have been evaluated, so the number of studies listed in Table 1 is more than the number of included articles.

Overall, the sensitivity of antigen-based methods for the diagnosis of human giardiasis ranged from 45% (95% CI: 31-59%) to 100% (95% CI: 100-100%) and the summary estimate of sensitivity for the antigen detection assays was 92% (95% CI: 90-93%).

The pooled estimated specificity was 97% (95% CI: 96-98%), ranged from 81% (95% CI: 68-89%) to 100% (95% CI: 98-100%). Positive and negative predictive values ranged between 45 % (95% CI: 31-59%) to 100% (95% CI: 100-100%) and 81% (95% CI: 68-89% to 100% (95% CI: 98-100%), respectively. The summary estimate of PPV and NPV were 92 % (95% CI: 90-93%) and 97% (95% CI: 96-98%) respectively.

Fig. 2 and 3 show the results of individual and pooled sensitivity and specificity estimates and Fig. 4 and 5 shows the PPV and NPV for the antigen detection assays for the diagnosis of human giardiasis.

Comparing the performance of the assays by region revealed a significant difference in the assay's performances in different regions of the world.

Based on the data extracted from the included articles, the most common target antigen for the serodiagnosis of giardiasis has been *G. lamblia*-specific antigen 65 (GSA-65). This 65-kDa glycoprotein is present in both cyst and trophozoite forms and is considered one of the best diagnostic antigens. The lowest and highest sensitivity and specificity of the GSA-65 antigen in different serological assays have been reported to be 81%-100% and 92%-99%, respectively.

Table 1: A summary of the included studies

	No. of subjects		*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*Diagnostic method*	*Ref.*
	Giardia patients	Controls						
1	76	60	98.7	100	100	96.4	ELISA	(19)
2	86	32	88	97	86	98	*CIE	(20)
3	86	32	94	95	76	97	ELISA	(20)
4	86	32	96	97	96	93		(20)
5	86	32	90	91	88	92	ELISA	(20)
6	80	220	92	87	86	96	ELISA	(21)
7	80	220	78	91	84	94	ELISA	(21)
8	21	283	100	99.2	95.2	100	ProSpecT/*Giardia* EIA test	(22)
9	30	17	84	96	67	98	Double antibody ELISA	(23)
10	85	77	95	82			ELISA	(24)
11	100	50	100	100	-	-	DFA - TechLab *Giardia*/Crypto IF kit	(25)
12	100	50	100	100	-	-	DFA - Merifluor *Cryptosporidium*/ *Giardia*	(25)
13	100	50	96	100	-	-	EIA - TechLab	(25)
14	100	50	99	100	-	-	EIA - Cambridge	(25)
15	100	50	98	100	-	-	EIA - Premier Meridian	(25)
16	100	50	94	100	-	-	EIA - Alexon	(25)
17	100	50	99	100	-	-	EIA - Trend	(25)
18	8	54	87.5	96.8	77.7	98.1	ProSpecT copro-antigen kit (Alexon,	(26)

							California)	
19	70	152	100	100	100	100	Alexon ProSpecT *Giardia* Microplate	(27)
20	70	152	100	100	100	100	Alexon ProSpecT	(27)
21	70	152	90	100	100	95.6	Alexon ProSpecT *Giardia* Rapid	(27)
22	70	152	95.7	100	100	98.1	Alexon ProSpecT	(27)
23	70	152	88.6	100	100	95	ELISA	(27)
24	70	152	92.9	100	100	96.8	Meridian Premier	(27)
25	70	152	98.6	99.3	98.6	99.3	Trend *G. lamblia* Direct Detection System	(27)
26	70	152	97.1	100	100	98.7	Trend *Giardia* Detection RS Test	(27)
27	30	60	100	95	90	100	ProSpecT *Giardia* Microplate Assay	(28)
28	77	479	96.1	98.5	91.3	99.3	Nonenzymatic rapid immunoassay (Alexon-Trend, Ramsey, Minn.).	(29)
29	56	112	91	99	98	96	EIA - Alexon	(30)
30	56	112	63	95	86	83	EIA - *Giardia* CELISA (Cellabs, Australia),	(30)
31	56	112	81	99	98	91	EIA - DSL-*Giardia*-ELISA (DSL, Germany)	(30)
32	56	112	81	96	92	91	EIA - Melotest Giardiasis Ag (Melotec, Spain)	(30)
33	30	73	96.3	98.7	-	-	EIA - Alexon	(31)
34	21	303	100	99.6	95.6	100	EIA (Ridascreen *Giardia*)	(32)
35	69	26	100	95	91	100	ELISA	(33)
36	170	231	93.5	100	100	95.5	ImmunoCard STAT!	(34)
37	32	214	90.6	99.5	-	-	EIA - ProSpecT	(35)
38	32	214	81.3	99.5	-	-	ImmunoCard STAT	(35)
39	106	104	97.2	100	100	97.2	(SIMPLE-READ *Giardia* rapid assay; Medical Chemical Corp.)	(36)
40	45	175	80	99.4	97	95.1	ICT - Rida Quick *Giardia*	(37)
41	45	175	80	100	100	95.1	ICT - Rida Quick Combi	(37)
42	45	175	82.2	98.9	94	95.6	EIA (Ridascreen *Giardia*)	(37)
43	45	175	44.4	100	100	87.4	ICT - Rida Quick *Giardia*	(37)
44	101	99	98	100	100	98	The rapid immunoassay (ImmunoCard STAT!	(38)
45	32	104	98.4	100	98	99.3	*Giardia*/*Cryptosporidium* Check test (TechLab, Inc.)	(39)
46	233	30	98.9	100	100	100	RIDASCREEN® *Giardia* ELISA test	(40)
47	233	30	89.6	93.8	96.8	93.8	Immunochromatographic *Giardia* test	(40)
48	233	30	92.2	96.8	98.5	96.8	Immunochromatographic	(40)
49	42	42	76.4	100	-	-	ELISA	(41)
50	23	243	100	100	100	100	The TriageMicro Parasite Panel	(42)
51	80	316	96.2	97.7	91	99	Immunochromatographic	(43)
52	31	68	96.8	99.5	100	99.2	Immunochromatographic (Single Crypto IC test)	(44)
53	31	68	96.8	99.5	100	99.2	Immunochromatographic (Crypto-*Giardia* Combo IC test)	(44)
54	31	68	96.8	99.5	100	99.2	Immunochromatographic (Crypto-*Giardia*-*Entamoeba* Triple IC Test)	(44)
55	31	68	93.5	97.7	90	98.4	ELISA	(44)
56	22	128	78.57	100	100	95.31	ELISA	(45)
57	22	128	84.62	100	100	96.87	DFA	(45)
58	21	127	95.2	99.2	95	99.2	The rapid immunoassay (ImmunoCard STAT!) Bioscience, Boxtel, The Netherlands)	(46)
59	48	40	72.9	100	100	75.5	EIA (Ridascreen *Giardia*)	(47)
60	48	40	93.8	100	100	93	EIA (Serazym *Giardia*)	(47)
61	41	42	95.12	92.85	92	95.12	Sandwich ELISA	(48)
62	45	155	93.3	99.4	-	-	Quik Chek (Techlab, Inc.).	(49)
63	50	70	100	95.71	94	100	*Giardia*/*Cryptosporidium* check test (TechLab, Inc.)	(50)
64	50	70	97.5	100	100	98.5	RIDA Quick *Giardia*	(50)
65	72	40	94.4	92	95	88	Sandwich ELISA	(51)

66	72	40	95.8	95	97	92.6	Nano-sandwich ELISA	(51)
67	80	55	94.12	94.83	96	91.67	Sandwich ELISA	(4)
68	1420	260	100	91.5	68	100	RIDASCREEN® *Giardia* ELISA test	(52)
69	32	38	79.3	100	-	-	ImmunoCardSTAT!	(53)
70	32	38	65.5	100	-	-	Crypto/*Giardia* Duo-Strip (Coris Bio-concepts, Gembloux, Belgium)	(53)
71	32	38	83.30	100	-	-	RIDA®QUICK *Cryptosporidi-um/Giardia/Entamoeba* Combi)	(53)
72	32	38	100	93.8	-	-	*Giardia*/Cryptosporidium Chek test (TechLab, Inc.)	(53)
73	38	32	100	95	-	-	*G. lamblia* ProSpecT ELISA Micro plate	(53)
74	12	78	100	96.15	80	100	Dia *Giardia lamblia* ELISA	(6)
75	12	78	100	94.87	75	100	ImmunoCard STAT	(6)
76	62	298	96.8	91.6	70	99.3	RIDASCREEN® *Giardia* ELISA	(7)
77	50	50	98	96	96	97.9	RIDASCREEN® *Giardia* ELISA	(54)
78	50	50	96	96	96	96	Immunochromatographic *Giardia* test	(54)
79	127	137	91	91	94	91	ELISA (NovaTec Immunodiagnostic GMBH ELISA kit, Germany)	(55)
80	27	146	63	96.6	77	93.4	ImmunoCardSTAT RDT	(56)
81	32	40	81.3	97.5	96	86.7	Dot-ELISA	(57)
82	32	40	96.9	97.5	96	97.5	NMB-Dot-ELISA	(57)
83	3	230	100	100	100	100	Crypto/*Giardia* K-SeT®	(58)
84	81	40	88	92	84	93.87	Sandwich ELISA	(59)
85	81	40	92	94	88	95.91	Nano-sandwich ELISA	(59)
86	30	66	70	88	87	71	ELISA	(60)
87	30	66	76.7	84	88	75	ICT - Rida Quick Combi	(60)
88	25	125	96	99.2	96	99.2	Immunochromatographic (Crypto-*Giardia* Combo IC test)	(61)

*CIE: counterimmunoelectrophoresis;

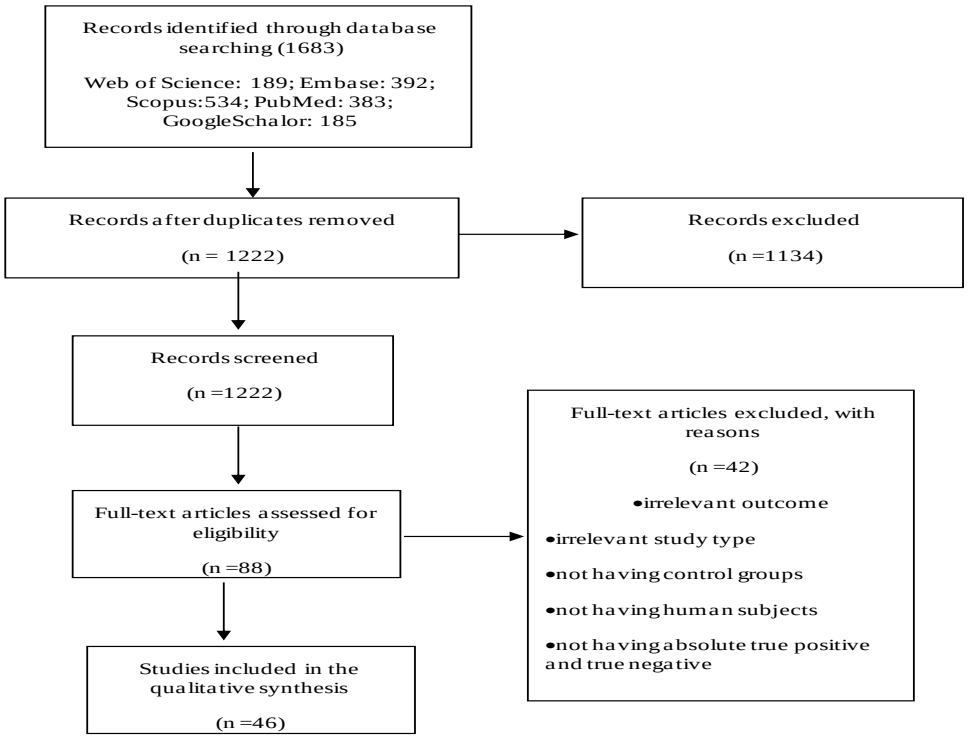

Fig. 1: The PRISMA flow diagram of the included studies

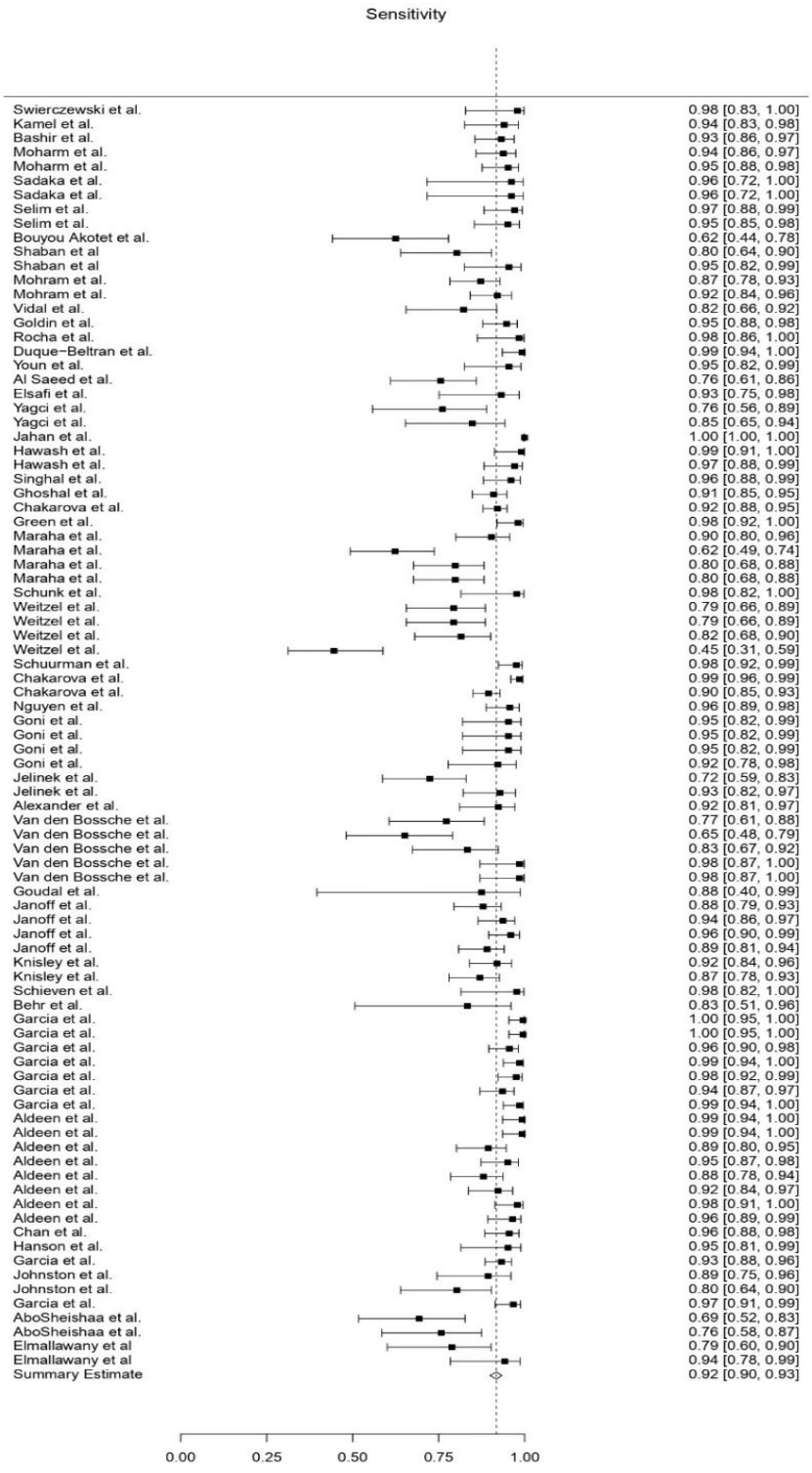

Fig. 2: Individual and pooled sensitivity estimates of antigen-based serological assays for the diagnosis of giardiasis. As presented in Fig. 2, the sensitivity analysis suggests an overall sensitivity of 92% (95% CI: 90-93%)

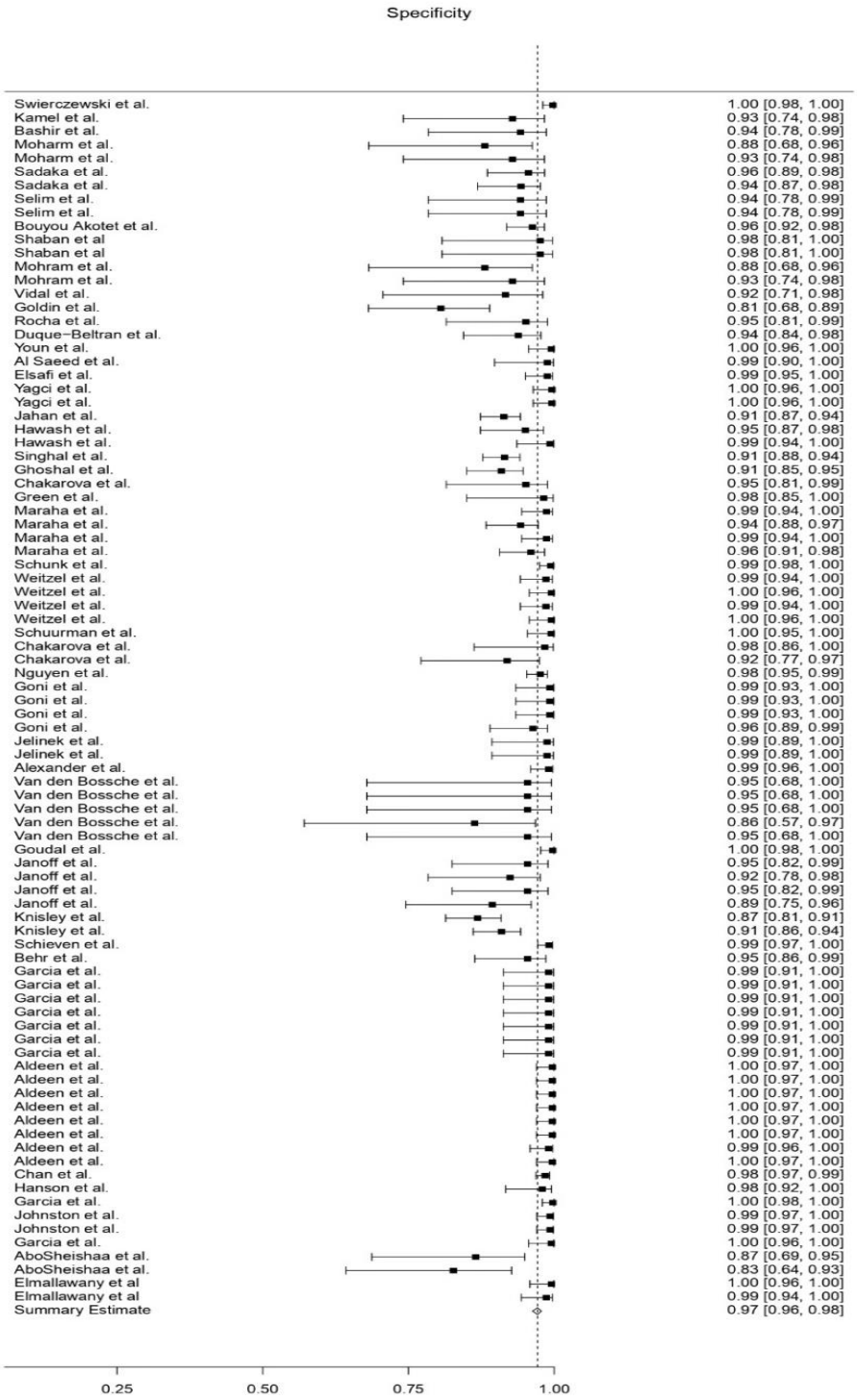

Fig. 3: Individual and pooled specificity estimates of antigen-based serological assays for the diagnosis of giardiasis. As presented in Fig. 3 the specificity analysis suggests an overall specificity of 97% (95% CI: 96%-98%)

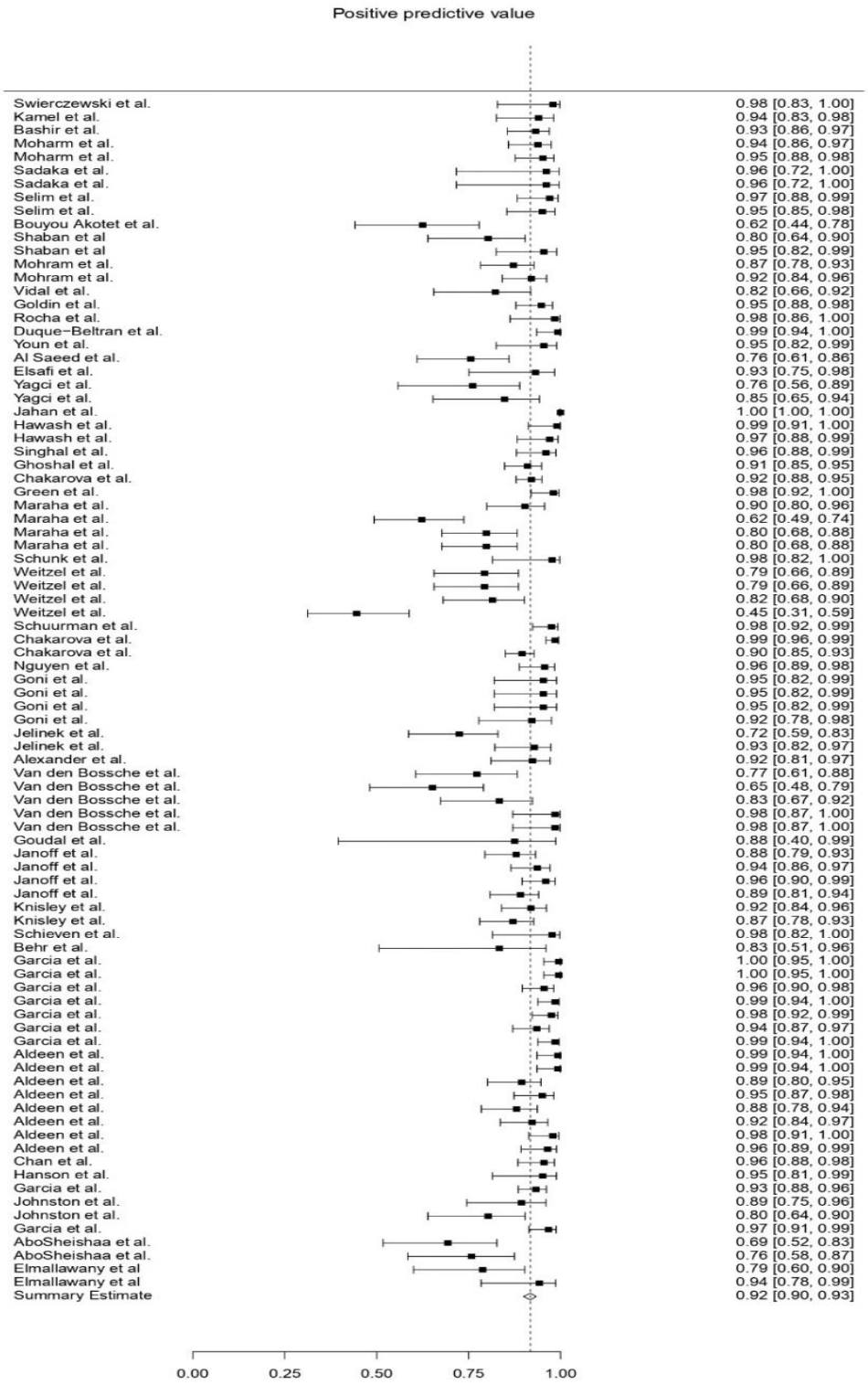

Fig. 4: Individual and pooled PPV estimates of antigen-based serological assays for the diagnosis of giardiasis. As presented in Figure 4, the PPV analysis suggests an overall PPV of 92% (95% CI: 90%-93%)

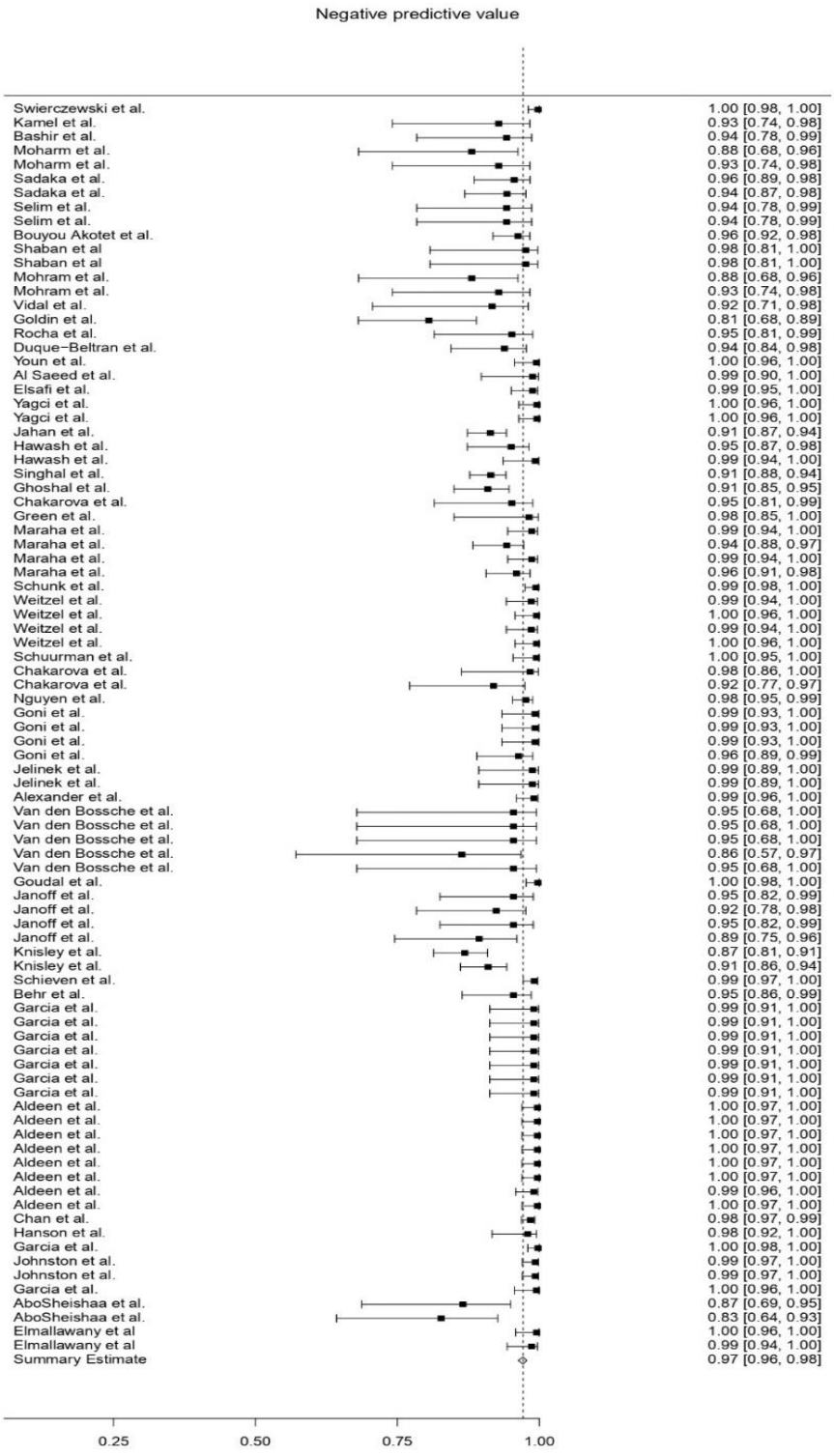

Fig. 5: Individual and pooled NPV estimates of antigen-based serological assays for the diagnosis of giardiasis. As presented in Fig. 5 the NPV analysis suggests an overall NPV of 97% (95% CI: 96%-98%)

Discussion

Various enzyme-linked immunoassays or rapid chromatographic tests with different performances have been developed for the detection of *Giardia* antigens in the fecal sample (27, 33, 36, 40, 62). Variation in diagnostic specificity of different assays can be due to cross-reactivity whereas discrepancy in sensitivity is linked to the intermittent shedding of cysts and *Giardia* antigens, using formalin as a fixative or a low number of cysts or trophozoites in the fecal sample.

Coproantigen detection methods can detect prepatent infections before the excretion of cysts or trophozoites in host feces. They can be used for rapid screening of large numbers of fecal samples. Unlike microscopy, coproantigen detection can be used for analyzing the preserved/frozen samples. In addition, the coproantigen-based method is useful for assessing the prevalence of the diseases in epidemics when a large number of samples need to be tested (48, 63). In addition to the above, through antigen-based methods, it is possible to identify simultaneously three important intestinal parasites, namely *Giardia, Cryptosporidium, and Entamoeba*, as the Tri-Combo test does. In two recent studies, reasonable sensitivity and specificity have been reported for the Tri-Combo test (5, 64). Moreover, sensitivities and specificities of currently available antigen-based tests for the diagnosis of giardiasis are reported to be; 63.6% and 96.6% for ImmunocardSTAT® C/ G (Meridian Bioscience Inc., USA), (65), 83%, and 100% for ImmunoCard STAT!® CGE (Meridian Bioscience Inc., USA) (53), 58% and 100% for *Cryptosporidium* and *Giardia* Duo-Strip (Coris BioConcept, Belgium), and 83% and 100% for RIDA®QUICK Combi (R-biopharm Diagnostic, Germany) (53).

Various factors may contribute to the efficacy of antigen-based diagnosis methods. The applied serological method, type of antigen, method of antigen preparation, antigen load in the patients, and geographical region, are important factors that may be effective in the performance of antigen-detection assays for the diagnosis of giardiasis. Based on the findings of the present meta-analysis, the sensitivity and specificity of antigen-based assays in the diagnosis of giardiasis are acceptable and satisfactory. The target antigen is one of the most important factors in developing an antigen-based detection assay for the diagnosis of giardiasis. The use of antigens in different phases of the *Giardia* life cycle (trophozoites or cysts) has been shown to have comparable results (20). On the other hand, antigen burden in the patients, which is often directly related to the clinical symptoms in patients, is an unavoidable factor in the results of antigen-based detection assays.

The findings of the present study documented that the performances of antigen-based tests in the diagnosis of giardiasis vary in different geographical areas. This difference can be largely due to the genotypic diversity of the parasite in different regions. The distribution of *Giardia* assemblages in different regions of the world and even in a given country has been reported in different studies and this may affect the efficacy of *Giardia* diagnostic tests in different regions (66-72). Not only the patients' region of residence is important in the obtained outcomes of the assays, but also the place of test execution (in terms of equipment, the experience of the operator, etc.) can be effective in increasing or decreasing the sensitivity or specificity of the test.

Most of the studies reviewed in this meta-analysis indicate the high specificity of antigen-based methods in the diagnosis of human giardiasis. This in turn indicates that the use of antigen-based methods significantly reduces the chance of cross-reactivity compared to antibody-based methods.

Conclusion

Overall, despite the differences in the patients studied, the geographical area of study, the type of antigen used, and the type of test, yet the antigen-based detection methods have acceptable and satisfactory performance in the diagnosis of human giardiasis. The task ahead is to identify more specific target antigens and design simpler, cheaper, and more sensitive methods for the diagnosis of this common worldwide-distributed parasitic infection.

Competing interest

The authors declare no competing interest.

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