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REVIEW

Immunodiagnosis of human hydatid disease: Where do we stand?

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Abstract

Cystic echinococcosis (CE) is a zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus*. Diagnosis of CE mainly relies on a combination of serological testing along with imaging approaches. A variety of serological methods, mainly based on hydatid cyst fluid, antigen B (AgB) and antigen 5, have been developed and used for immunodiagnosis of CE, yet their performances are not satisfactory. Although utilizing of recombinant or synthetic antigens, improved the performance of serological tests, it has not applicably overcome the problem of low sensitivity and cross reactivity, seen in the diagnosis of CE. Performances of immunodiagnostic tests based on AgB subunits are promising. The 8 kDa subunit of AgB is the most studied antigen in native, synthetic or recombinant form for diagnosis of CE. From the 5 subunits of AgB, antigen B8/1 and B8/2 provided the highest diagnostic sensitivity and specificity. Moreover, detecting of specific antibodies of IgG subclasses has improved the efficacy of immunodiagnostic tests. Among the IgG subclasses, both IgG2 and IgG4 are considered as good markers for diagnosis and IgG4 as a suitable marker for follow up of the patients. In this review an overview of immunodiagnostic methods, related antigens and their performances in the diagnosis of CE are given. The paper highlights pitfall and challenges in the serological diagnosis of CE. Moreover, limitation of currently available immunodiagnostic tests and the most recent development in the designing and application of serological assays for diagnosis of CE in human are addressed.

Key words: Immunodiagnosis; Cystic echinococcosis; Hydatid cyst

Core tip: Cystic echinococcosis (CE) (hydatid cyst) is one of the most important parasitic diseases, causing tremendous morbidity and mortality for the human patients. Diagnosis of CE mainly relies on ultrasound images of the cyst along with serological testing. So far, there is no highly specific and sensitive immunodiagnostic test for diagnosis of CE

and performances of the currently available tests are not satisfactory. Different antigenic sources including hydatid cyst fluid, antigen B and 5, excretory-secretory antigens of larval stage or adult worm have widely been used for development of serological assays for diagnosis of CE. Utilizing of antigen B subunits in immunodiagnostic tests and detection of IgG subclasses, as a good marker, opened a promising perspective in diagnosis of this debilitating disease.

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INTRODUCTION

Cystic echinococcosis (CE), known as hydatid cyst or hydatid disease, is a zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*). Dogs and other canids harbor the adults tape worm and herbivores acts as intermediate host and become infected through ingestion of parasite's eggs. Human acquire the infection by accidental ingestion of *E. granulosus* eggs.

CE with its significant economic and medical impact constitutes an important public health problem in many developing countries[1-3]. An estimated 1.2 million people worldwide are affected by CE and the disease accounts for annual estimate of 3.6 million DALYs (disability adjusted life years) through the world[4]. Early and proper diagnosis of CE can provide appropriate management and suitable treatment of the disease[5].

Diagnosis of CE is mainly confirmed through a combination of relevant history, serological testing, along with imaging approaches. A variety of serological methods have been developed and used for immunodiagnosis of CE in recent years, including indirect hemagglutination (IHA), immunoblotting, enzyme-linked immunosorbent assay (ELISA), indirect fluorescent-antibody (IFA), latex agglutination test, and immunochromatography test[1,6-11]. For the development of these assays different antigens from adult worm, protoscolices, worm eggs or hydatid cyst fluid have been defined, purified and evaluated in the aforementioned serological tests.

Diagnosis of CE has drastically improved during the last two decades. Progress in methods for antigen purification, cloning expression and purification of *E. granulosus* recombinant antigens, and defining and synthesis of immunodominant peptides contributed to this development. Nevertheless, immunodiagnosis of CE is still problematic. Commercially available serological tests show unsatisfactory performance. The lack of standardization of immunodiagnostic assays and also antigen preparation contribute to discrepancy in results reported in different laboratories. Cyst size, stage and location as well as patients characteristics may be accounted for the discrepancy of the same test performance in different clinical diagnostic laboratories.

Hence, serological assays still have a complementary role to imaging in the diagnosis of CE. Low sensitivity (up to 30% of false negativity) and also low specificity (up to 25% of false positivity) make serological results difficult to interpret[12-17].

Pitfalls and challenges in the diagnosis of CE

In spite of the development of a variety of immunodiagnostic test, following diagnostic pitfalls and challenges still exist in the diagnosis of CE.

Available immunodiagnostic tests give a relatively high rate of false-negativity. False negative results in immunodiagnostic tests for CE may be seen in patients with small cysts, intact cysts, cysts in extrahepatic locations, heavily calcified cysts (*e.g.,* non-viable), or cyst in privilege sites (brain or eye). Akbulut *et al*[18] reported that 15 out of 40 patients with pancreatic echinococcosis, found in the literature have had negative serological testing for CE. Among 65 CE patients in Germany, false negative serological results were reported in 18% by IHA and in 15% by ELISA[19]. In a study by Akcam *et al*[20] more than 20% of patients with extra-hepatic cysts were reported to be negative by IHA test. Using WB, 10 cases of IHA-negative were found to be positive. In a study by Wuestenberg *et al*[21], CE was confirmed in 9 cases of IHA-negative by clinical findings and imaging (US). Cardiac hydatid cyst, with 54 mm × 45 mm size, was serologically negative in Canpolat *et al*[22] report. Karakasli *et al*[23] reported a case of large spinal-para spinal hydatid cyst with negative ELISA and WB testing. They suggested that clinical and neuro-radiological findings should be considered in such cases. Review of 100 case of pulmonary hydatidosis by Zapatero *et al*[24] revealed that positive serological test have been present with ruptured cyst (positive IHA in all of ruptured cyst) while the test detected only 80% of patients with unruptured cysts. Serological test for CE have been negative in human immunodeficiency virus (HIV)-positive cases[25].

Currently available tests give rather high rate of false-positive reaction in patient infected with other parasites (notably cestodes) or even in healthy subjects. False positive results are related to cross reactant antibodies.

Differentiation of past (cured or calcified cyst) from present (active or progressive) hydatid infection is difficult by existing antibody detection assays. Antibody titer may remain for years, even after surgical removal of the cyst or proper drug treatment[26,27]. Therefore a positive serological test may not necessarily imply the presence of active cyst or even the reactivation of CE.

Hydatid cysts in unusual locations may complicate its diagnosis. Congenital, choledochal and pancreatic pseudocysts along with lipoma, ovarian intra-abdominal cystadenoma and intra-hepatic haematoma may be misdiagnosed as hydatid cyst by ultrasonography/computerized tomography (US/CT)[13]. In all of these

conditions an appropriate serological test would be quite helpful with negative results.

Performance of serological tests varies in different pathological stage of CE according to WHO classification[28].

A single defined molecule may not be sufficient for diagnosis of CE. Recent immunoproteome analysis of hydatid cyst fluid (HCF), in different stages of cyst (based on WHO classification), revealed that specific immunodominant epitopes changes from[29] one stage to another stage. This indicates that more than one defined immunodominant antigen may be needed to diagnosis CE in different status of the cyst.

Antigenic sources for immunodiagnosis of CE

Antigenic sources which have widely been used for immunodiagnosis of CE are HCF, component of HCF, ES of protoscolices or adult worm, and also extract of adult worm or larval stage. Antigen for immunodiagnosis of CE has been comprehensively reviewed by Carmena *et al*[12]. The main antigens for diagnosis of CE and their performance in diagnosis of CE are discussed below.

HCF: HCF is the most common antigenic source which has been used for diagnosis of CE. HCF is a mixture of host (albumin, globulins) and parasite components[30-32]. Sensitivities of serological tests based on HCF are high but their specificities are far from satisfactory (30%-90%)[8,10,12,28,31,33-35]. Many of available commercial kits are using HCF in ELISA system for diagnosis of CE. Table 1 summarizes the performance of HCF in diagnosis of CE in different serological assays.

Using HCF as a source of antigen, Sedaghat *et al*[35] evaluated the performance of a simple dot ELISA and CCIEP (counter current immunoelectrophoresis) for diagnosis of human hydatidosis and found a sensitivity and specificity of 100% and 89.1% for Dot-ELISA and 80% and 62% for CCIEP. Dot ELISA had a better performance in comparison with CCIEP. Using HCF, EL-Shazly *et al*[8] reported a sensitivity of 96.7% and specificity of 97.5% for ELISA and 86.7% and 95% for IHA. Al-Sherbiny *et al*[6] applied the camel HCF in a dipstick assay and reported a high diagnostic sensitivity (100%) and specificity (91.4%).

CCIEP is a relatively sensitive, but not specific method for diagnosis of CE. In a retrospective study conducted by Sadjjadi *et al*[15] hospital records of 1227 surgically proven CE cases were examined and found that only 62% of cases had a CCIEP positive test in comparison with 96.3% of positive findings by US and pathology.

Antigen 5: Antigen 5 (Ag5) is one of the most immunogenic and abundant part of HCF. It composed of 57 and 67 kDa components and dissociate into 38 and 22-24 kDa subunits under reducing conditions[36]. Ag5, after AgB, is one of the most studied antigens in the serodiagnosis of CE. Numerous studies pointed out that Ag5 has a high rate of cross-reactivity with sera of healthy controls or other non-CE patients[12,17,37]. Its performances in native, recombinant or synthetic forms have not been satisfactory due to either low sensitivity (50-54), or specificity because of cross reactivity with sera of the patients with other cestoda, trematoda or even nematoda. In an Ag5-based ELISA, Khabiri *et al*[38] reported that IgE and IgG4 are the most important antibodies, with low cross reactivity with sera of healthy control and non-CE cases.

Contrary to these reports, a recent study by Pagnozzi *et al*[39] demonstrated that highly enriched Ag5, by chromatographic method, attained highly specific and unambiguous results, in Western blotting and ELISA system in diagnosis of CE. The authors indicated that low performance of this antigen in previous studies is related to non-properly purified antigen which have been used and considered that highly purified Ag5 is a promising antigen in diagnosis of CE. Having said that, the low number of sera tested in their study does not allow drawing a decisive conclusion. Table 2 shows the performance of Ag5 in diagnosis of CE in different serological assays.

Antigen B: Antigen B (AgB) is a thermostable polymeric lipoprotein of 120-160 kDa, composed of 8 kDa subunits which dissociates into 8/12, 16 and 24 kDa subunits, under reducing condition in SDS-PAGE[36]. AgB is considered as the main antigen of HCF with high specificity and sensitivity in serological diagnosis of CE[9,10,31,40-42].

AgB is highly immunogenic, a feature that makes this antigen a suitable candidate for immunodiagnosis of CE. The smallest subunit, 8 kDa, considered as the most appropriate antigen in diagnosis of CE. Not surprisingly, the 8 kDa subunit of AgB is the most studied antigen in native, synthetic or recombinant form for diagnosis of CE. Sarkari *et al*[42] obtained diagnostic sensitivity and specificity of 100% and 80% when AgB was evaluated in an immunoblotting system. In their study from 40 sera of hydatidosis patients, 32 cases (80%) detected the 8 kDa subunit, 29 cases (72.5%) recognized the 16 kDa component and 29 cases (72.5%) detected the 24 kDa subunit of antigen B. In continuation of their study, when the AgB was used in an ELISA system, sensitivity of the system was determined to be 92.5% and the specificity was found to be 97.3%[10].

Recombinant AgB are not doing much better in diagnosis of CE when compared with native homologues antigens. The performance of rAgB subunits for diagnosis of CE was evaluated by Jiang *et al*[40] where they reported performance order of AgB1 > AgB4 > AgB2 > AgB5 > AgB3. It was found that in some cases antibodies against subunits of AgB was not produced. In another study, Jiang *et al*[43] reported that AgB1 has higher diagnostic sensitivity in comparison with AgB2 and AgB4. However, in Virginio *et al*[44] study, antigen B8/2 provided the highest diagnostic sensitivity (93.1%) and specificity (99.5%) in ELISA system. In Leggatt *et al*[45] study, a sensitivity of 90.9% was reported for the 12 kDa subunit of AgB (corresponding to the smallest

Table 1 Performance of hydatid cyst fluid in diagnosis of cystic echinococcosis in different serological assays

Antigen	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reactions	Year	Ref.
	CE patients	Other disease	Healthy control						
SHCF	78	24	15	IgG ELISA	72.4		NR	2001	[71]
BHCF	129	65	203	IgG ELISA	77.6	96.6	Cysts, Toxoc.	2003	[44]
CHCF	26	35	10	Dipstick assay	100	91.4	Cysts, AE, Trichinosis, Schist., Fascio	2004	[6]
CHCF	26	35	10	EITB	100	91.4	Cysts, AE, trichinosis, Schist, Fascio	2004	[6]
CHCF	26	30	10	IgG ELISA	96.2	100	None	2004	[6]
SHCF	102	68	95	IgG ELISA	88.2	80.9	AE, Cysts, Schist, Fascio, Taeniasis, Dirofilariasis	2008	[72]
SHCF	120			Casoni's skin test	88.2	80.9	NR	2005	[73]
SHCF	120			Casoni's skin test	70	87	NR	2005	[73]
SHCF	120			Casoni's skin test	62	85	NR	2005	[73]
SHCF	120			IHA	56	84	NR	2005	[73]
SHCF	25	15	25	ELISA on serum	72	76	Cysts, Ascaris, Ambs liver abscess	2007	[74]
SHCF	25	15	25	ELISA on urine	84	76	Cysts, Ascaris, Ambs liver abscess	2007	[74]
SHCF	25	15	25	ELISA on saliva	56	76	Cysts, Ascaris, Ambs liver abscess	2007	[74]
SHCF	40	40	70	CCIEP	97.5	58.1	Fascio, Toxoc, Taenia, Malignancies	2007	[10]
SHFF	204	53	90	IEP	31	100	None	2000	[75]
SHFF	204	53	90	IHA	54	100	None	2000	[75]
SHFF	204	53	90	IB	80	96	Cysts, Serous cysts	2000	[75]
SHCF	35	12	25	Dot-ELISA	100	89.1	Ascaris, Taenia, Strogyl	2010	[1]
SHCF	35	12	25	CCIEP	80	62	Ascaris, Strogyl, Toxop	2010	[1]
SHCF	59	60	39	IgG ELISA	91.5	96	Clonorchiasis	2013	[76]
hHCF	50	15	20	IB	83	98	None	2014	[28]
hHCF	50	15	20	IgG IB	83	98	None	2014	[28]
SHCF	50	40	20	IgG ELISA	92	85	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgM ELISA	70	93.33	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgE ELISA	86	96.66	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG1 ELISA	82	98.33	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG2 ELISA	74	95	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG3 ELISA	52	36	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG4 ELISA	86	28	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
Psx Ag	113	112	121	DIGFA	87.6	90.90	Hd, Cysts, HCC, HH	2015	[48]
CPsx extract	147	88	60	IgG ELISA	90	57	AE, Trypanosomiasis	2002	[76]
Emwl Ag	50	154		WB IgG	98		NCC	2000	[14]
Emwl Ag	50	154		IHA > 80	94.3		NR	2000	[14]
Emwl Ag	50	154		IHA > 320	80		NR	2000	[14]
Emwl Ag	50	154		IgG ELISA	79.4		NR	2000	[14]

EITB: Enzyme linked immunoelectrotransfer blot; IHA: Immune hemagglutination assay; CHCF: Camel hydatid cyst fluid; SHCF: Sheep hydatid cyst fluid; hHCF: Human hydatid cyst fluid; SHFF: Sheep hydatid fluid fraction; Hd.: Hepatic distomiasis; Emwl Ag: Whole larval antigen from *Echinococcus multilocularis*; CPsx extract: Crude protoescolex extract; Psx Ag: Protoscoleces antigen; BHCF: Bovine hydatid cyst fluid; Ascaris: Ascariasis; Toxop: Toxoplasmosis; Fascio: Fascioliasis; Cysts: Cysticercosis; Ambs: Amebiasis; Toxoc: Toxocariasis; Schist: Schistosomiasis; AE: Alveolar echinococcosis; Strongyl: Strongyloidiasis; HH: Hepatic hemangioma; NR: Not reported; CE: Cystic echinococcosis; ELISA: Enzyme-linked immunosorbent assay.

subunit of AgB) in a blotting system. More than 5% (5.5%) of cysticercosis patients reacted with this subunit.

The 12 kDa subunit of AgB, was cloned and expressed by Abdi *et al*[46]. The antigen was comparatively evaluated for diagnosis of CE, with native AgB and HCF. The sensitivity and specificity of rAgB, in ELISA system was similar to HCF (96% and 97%), and lower than native AgB (98.6% and 100%).

A recombinant antigen of B8/1 (rAgB), showed a high sensitivity (94.6%) and specificity (93.9%) for diagnosis of CE, using serum samples from Iran, China and Japan, in comparison with HCF, native AgB, prepared from sheep HCF, either from Iran of Japan[31].

Mamuti *et al*[41] cloned and produced recombinants of EmAgB8/1 from *E. multilocularis* and EgAgB/1 from

Table 2 Performances of antigen 5 for immunodiagnosis of cystic echinococcosis in different serological assays

	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reaction	Year	Ref.
	CE patient	Other disease	Healthy control						
Ag5	39	51	29	IgG ELISA	54	89	AE	2000	[9]
Ag5	58	36	40	IgG ELISA	100	70.17	Leish, Toxop, Fascio	2006	[39]
Ag5	58	36	40	IgG1 ELISA	100	70.17	Leish, Toxop, Fascio	2006	[38]
Ag5	58	36	40	IgG4 ELISA	75.8	93.02	Toxop, Fascio	2006	[38]
Ag5	58	36	40	IgE ELISA	70.1	100	None	2006	[38]
rAg5	34	36	18	IgG ELISA	65	89	AE, Cysts	2005	[77]
rAg5-38s	34	36	18	IgG ELISA	21	97	AE	2005	[77]

Leish: Leishmaniasis; Toxop: Toxoplasmosis; Fascio: Fascioliasis; Cysts: Cysticercosis; AE: Alveolar echinococcosis.

E. granulosus and evaluated their antigenic reactivity in Western Blotting and ELISA in comparison with that of counterpart, an 8 kDa subunit of AgB. WB showed reactivity with 81.3% of sera from CE patients and 40.6% of sera from alveolar echinococcosis (AE) patients, while EgAgB8/1 showed reactivity with 86% of CE and 42% of AE patients. Both EmAgB/1 and EgAgB/1 showed similar reactivity with 37.8% of sera from AE and 88% of sera from CE patients.

A synthetic P176 peptide related to N-terminal extreme of AgB/1 subunit yielded a sensitivity and specificity of 78.69 and 96.88 for pulmonary hydatid cyst[47].

Application of antigen B in a dot immunogold filtration assay increased the test specificity (98.3%) but in turn decreased the sensitivity (77.9%) of the assay, compared to native antigen[48].

Source of antigen B is an important factor which affects the performance of the test for diagnosis of CE. In agreement with this, Rahimi *et al*[49] showed that AgB isolated from human and sheep liver cyst have the best performance in diagnosis of CE when compared with those antigen obtained from liver or lungs cyst of goat, cattle or camel.

Combination of antigen B and antigen 5 may increase the sensitivity of the test as currently used in a commercially available test. The commercially available Rapid Immunochromatography test VIRapid® HYDATIDOSIS test (Vircell, Spain) using antigen 5/B was evaluated by Tamer *et al*[50] for diagnosis of CE where they reported a sensitivity of 96.8% and specificity of 87.5%. In their study, the antigen cross reacted with sera from taeniasis and leishmaniasis patients and also a few (4%) of healthy controls.

Nature and quality of antigen B, isolated from HCF, may be variable based on the host species, cyst location, cyst status and also parasite strain. This is one of the reasons that different laboratories attain different results using AgB in serodiagnosis of CE. In view of this point, discrepancies in results of serodiagnosis of CE, using antigen B might be related to, method of antigen preparation, variation in host and strain of parasite, differences in antigen B, site of the cyst, clinical status and type of the cyst. Table 3 shows the performances of antigen B in diagnosis of CE in different serological assays.

Protoscolices antigens: Native metacestode-derived antigens show substantial (mainly more than 90%) sensitivities in diagnosis of CE[51]. However cross-reactivity with other parasitic diseases (fascioliasis, schistosomiasis, amebiasis, taeniasis, cysticercosis and filariasis) is the main drawback of using such antigens for serodiagnosis of CE. The best performance for serological tests of ELISA, IHA and IFA, was achieved for ELISA (87.5% sensitivity and 100% specificity), using metacestode antigen[52].

Detection of IgG subclasses: Detecting of specific antibodies of IgG subclasses may improve the diagnostic performance of immunodiagnostic tests. Xu *et al*[53] examined the seroreactivity of 42 IgG negative (total IgG) with IgM, IgE, IgA, and IgG subclasses and found that 32 cases were positive with either one or combined of two of other antibodies. The best seropositivity (42.95%) was reported with either IgG1 alone or a combination of IgG1 + IgA + IgM. IgG subclasses is usually linked to the status of cyst development. Findings of Daeki *et al*[54] demonstrated that IgG antibody response is associated with the growth and development of cyst, while IgG1, 2 and 3 responses are predominantly related to involutive phase in CE cysts. Patients with relapsing disease have a high level of IgG4 titer.

Lawn *et al*[55] demonstrated that concentration of CE-specific IgG subclasses (IgG1-4), are much correlated with disease activity than total IgG. Among the IgG subclasses, IgG2 provided the best correlation with clinical outcome. In a lateral fellow dipstick test, a sensitivity of 95% and specificity of 100% was reported for detection of IgG4, in comparison with IgG dipstick with 87.5% specificity[56]. Detection of antibodies mainly IgG subclasses (IgG1, 4) in urine of CE patients provide a similar result in comparison to serum sample in Chirag study[57].

Antigen detection for immunodiagnosis of CE

Antigen detection has been used for diagnosis of a few of parasitic diseases with satisfactory results[58-61]. Antigen detection might be useful for detection of current infection and also post treatment follow up of CE patients. However results with detection of hydatid cyst antigen

Table 3 Performances of antigen B in diagnosis of cystic echinococcosis

Antigen	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reactions	Year	Ref.
	CE patients	Other disease	Healthy control						
nAg B	204	21	90	IB	66	100	None	2000	[75]
nAg B	59	55	15	IgG ELISA	80	77	AE, NCC	2005	[77]
nAg B	90	86	27	IgG ELISA	77	85	AE, RA	2000	[9]
nAg B	204	21	90	IgG ELISA	74	100	None	2000	[75]
nAg B	31	87	29	IgG ELISA	77.41	81.9	AE, Ev, Schist, Toxoc	2000	[78]
nAg B	78	24	15	IgG ELISA	93.5	89.7	Distomatosis, Schist	2001	[71]
nAg B	129	65	203	IgG ELISA	60.3	92.6	Cysts,Toxoc	2003	[44]
nAg B	22	12	4	WB	77	100	Toxoc, Other cestodes	2010	[30]
nAg B	40	40	70	IgG ELISA	92.5	97.3	Fascio	2007	[10]
nAg B	40	40	70	CCIEP	97.5	58.2	Fascio, Toxoc, Taenia, Malignancy	2007	[10]
nAg B	204	53	90	IB	66	100	None	2000	[75]
nAg B	204	53	90	IgG ELISA	74	100	None	2000	[75]
nAg B	35	29	25	IgG ELISA	94.2	81.6	NR	2009	[16]
nAg B	55	72	50	IgG ELISA	96.4	97.2	None	2014	[68]
nAg B	113	112	121	DIGFA	92.9	81	HD, Cysts, HCC, HH	2015	[48]
Goat liver Ag B	47	30	40	IgG ELISA	91.4	92.8	NR	2011	[49]
Human liver Ag B	47	30	40	IgG ELISA	97.8	97.1	NR	2011	[49]
Bovine lung Ag B	47	30	40	IgG ELISA	78.7	85.7	NR	2011	[49]
Sheep lung Ag B	47	30	40	IgG ELISA	93.6	88.5	NR	2011	[49]
Camel lung Ag B	47	30	40	IgG ELISA	93.6	90	NR	2011	[49]
Sheep liver Ag B	47	30	40	IgG ELISA	95.7	92.8	NR	201	[49]
rAgB	204	21	90	IB	72	100	None	2000	[75]
rAgB	113	112	121	DIGFA	77.9	98.3	None	2015	[48]
rAgB8/1	31	87	29	IgG ELISA	54.84	80.17	AE, Schist, Toxoc	2000	[78]
rAgB8/1	129	65	203	IgG4 ELISA	91.4	91.7	Cysts	2003	[44]
rAgB8/1	59	55	15	IgG ELISA	68	88	AE, NCC	2005	[80]
rAgB8/2	31	87	29	IgG ELISA	83.87	98.28	Schist, Toxoc	2000	[78]
rAgB8/2	129	65	203	IgG ELISA	93.1	99.5	Cysts, Toxoc	2003	[45]
rAgB8/2	129	65	203	IgG4 ELISA	69	87.5	Cysts	2003	[45]
rAgB8/2	59	55	15	IgG ELISA	45	86	AE, NCC	2005	[77]
rAgB8/1	129	65	203	IgG4 ELISA	91.4	91.7	Cysts	2003	[44]
rAgB8/1	59	55	15	IgG ELISA	68	88	AE, NCC	2005	[77]
rAgB8/2	31	87	29	IgG ELISA	83.87	98.28	Schist, Toxoc	2000	[78]
rAgB8/2	129	65	203	IgG ELISA	93.1	99.5	Cysts, Toxoc	2003	[44]
rAgB8/2	129	65	203	IgG4 ELISA	69	87.5	Cysts	2003	[44]
rAgB8/2	59	55	15	IgG ELISA	45	86	AE, NCC	2005	[77]
B1t	102	68	95	IgG ELISA	83.3	87.5	AE, Schist, Cysts, Fascio,	2008	[9]
B2t	102	68	95	IgG ELISA	91.2	93	Cysts, Schist, Fascio	2008	[9]
2B2t	186	174	110	IgG ELISA	87.6	99.1	AE, NCC, Hepatitis	2012	[9]
rEgAFFPt	129	65	203	IgG ELISA	58.6	95.6	Cysts, Toxoc	2003	[44]
rEgCaBP2	129	65	203	IgG ELISA	84.5	96.6	Cysts, Toxoc	2003	[44]
rEgcMDH	129	65	203	IgG ELISA	89.7	95.1	Cysts	2003	[44]
rEgAFFPf	129	65	203	IgG ELISA	69	89.7	Cysts, Toxoc	2003	[44]
rEpC1-GST	324	502	70	IgG IB	92.2	95.6	AE, NCC, Schist, Liver cancer	2003	[79]
rTPxEg	100	218	20	IgG IB	39	69.3	AE, NCC	2004	[78]
rEgG5	23	138	20	IgG IB	61	70	AE, Cysts	2004	[79]
E14t	102	68	95	IgG ELISA	35.3	91.7	Schist	2008	[9]
C317	102	68	95	IgG ELISA	58.8	80.9	AE, Cysts, Taeniasis, Schist,	2008	[9]
p65	90	86	27	IgG ELISA	44	96	AE, Schist, Toxoc	2000	[9]
p175	90	86	27	IgG ELISA	49	94	AE, Schist, Toxoc	2000	[9]
p176	90	86	27	IgG ELISA	80	93	AE, Schist, Toxoc, Syph, Chagas	2000	[9]
p177	90	86	27	IgG ELISA	38	92	AE, Toxoc, Syph, Chagas	2000	[9]
pGu4	90	86	27	IgG ELISA	18	98	AE	2000	[9]

Leish: Leishmaniasis; Toxop: Toxoplasmosis; Fascio: Fascioliasis; Cysts: Cysticercosis; Ascaris: Ascariasis; Syph: Syphilis; Ambs: Amebiasis; Toxoc: Toxocariasis; Schist: Schistosomiasis; NCC: Neurocysticercosis; AE: Alveolar echinococcosis; nAg B: Native antigen B; rAgB: Recombinant antigen B; Ev: Ev: Polycistic hydatid disease (*E. vogeli*); RA: Rheumatoid arthritis; HCC: Hepatocellular carcinoma; NR: Not reported; CE: Cystic echinococcosis; ELISA: Enzyme-linked immunosorbent assay.

Table 4 Performances of antigen detection assays in immunodiagnosis of cystic echinococcosis

Antigen	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reaction (%)	Year	Ref.
	CE patient	Other disease	Healthy control						
Urinary antigen	40	24	25	Co-A	50	89.09	12.5	2000	[62]
Serum antigen	40	24	25	Co-A	73.08	94.23	12.5	2000	[62]
Serum antigen	35	29	25	IgG ELISA	25.7	98	3.4	2009	[16]
Serum antigen	141	25	25	LAT	72	98	4	2003	[7]
Serum antigen	40	24	25	CIEP	45	100	None	1997	[80]
Urinary antigen (ucon)	40	24	25	CIEP	22.5	95.91	8.33	1997	[80]
Urinary antigen (con)	40	24	25	CIEP	47.5	95.91	None	1997	[80]

ucon: Unconcentrated; con: Concentrated; CE: Cystic echinococcosis; LAT: Latex agglutination test; Co-A: Coagglutination; CIEP: Countercurrent immunoelectrophoresis; ELISA: Enzyme-linked immunosorbent assay.

for detection of CE are far from satisfaction[7,11,62-64].

Antigen detection in CE is much less sensitive than antibody detection and the later remains the most commonly used approach for diagnosis of this disease. Antigen can be detected in sera of 35%-85% of CE patients depends on the status and location of the cyst[7,16,63]. In some cases of CE circulating antigen has been detected in sera of patients who had not shown anti-hydatid antibodies in their serum. Swarna *et al*[11] reported a sensitivity of only 53.33% and specificity of 96.66% in a Dot-ELISA system for detection of hydatid cyst antigen in urine samples. Lower sensitivity (29.68) was obtained when CCIEP was used for detection of hydatid urinary antigen[62].

Using coagglutination test, a sensitivity of 47.5% was achieved for detection of hydatid antigen in urine[63]. Several interfering factors have been proposed to explain the poor performance of antigen-detection assays in diagnosis of CE. Among them are formation of immune complexes and low availability of free antigen, sequestration of antigen due to cyst layers, especially in intact cyst, and presence of interfering component in serum or urine, as demonstrated in other studies[58]. Cysts in privileged sites (*e.g.,* eye and brain) do not release enough antigens to be detected by serological assays.

Location of the cyst is an important issue in diagnosis of CE as one study pointed out that CE antigen can be detected in 46% of patients with liver cyst but not in any of patients with lung hydatid cyst[16]. In an attempt to develop an antigen detection assay for diagnosis of CE, Sadjjadi *et al*[16] evaluated an ELISA system for detection of circulation antigens in serum of CE patients. In their study, antigen was detected in only 9 out of 35 (25.7%) of cases. Table 4 summarizes the performance of antigen detection assays in diagnosis of CE.

Post treatment follow up: CE patients need to be followed up after treatment, to make sure about the risk of recurrence. Anti-CE antibodies may persist for several years after treatment[55]. Although antigen detection might be a useful approach in post-treatment follow-up, however its low sensitivity hampered its use for patients' follow-up. Different serological assays have been used for monitoring of surgically or chemically treated CE patients[26,64-66].

In a recent cohort study, CE patients were followed for a mean of 6 years and the level and isotypes of antibodies were evaluated before and after surgical or anti-helminthic drugs treatment. Results demonstrated that IgE, IgG1 and IgG4 are the most important antibodies for serological diagnosis of active CE. During post-operation, IgM, IgE, IgG1, IgG2 and IgG4 were the best correlative with disease activities[67]. Reiterová *et al*[68] reported that antibodies to AgB was not detectable three months after treatment but antibodies to HCF were remained detectable.

It has been reported that subclasses of IgG have different performance in diagnosis of primary in comparison to relapse cases of CE. One study suggested IgG2 as a good marker for primary infection and total IgG for detection of relapse cases[69].

Recombinant P29 protein of *E. granulosus* was synthesized by Ben Nouir *et al*[64] and evaluated for post-surgical follow-up of CE patients, in an ELISA and WB systems. Results indicated that, using P29-ELISA, all of initially seropositive cases of CE seroconverted to negative within three years after treatments, while HCF-ELISA remained positive in 90% of cases. Western Blotting, using P29, remained positive in only 10% of cases after 3 years while HCF-WB remained positive in more than 25% of cases after 3 years of follow-up. However the performance of P29 in initial diagnosis of CE has not been satisfactory.

In another study by this group, somatic protoscolex antigens of *E. granulosus* have been assessed for follow-up of surgically treated CE patients and found that only 29% of treated patients reaching seronegativity after 5 years of follow up. The conventional HCF-ELISA becoming negative in 15% of cases at the end of the follow up period[65].

A double 27 and 28 kDa antigen, in WB, was also reported as useful antigen for the follow up of CE

patients. However such bands were only detectable in 75% of the patients before treatment[65]. The prognostic value of AgB subunits was evaluated by Ben Nouir *et al*[64] in ELISA and WB systems. Patients were grouped into either cured or non-cured CE patient. Findings of the study showed that ELISA remained positive 4-5 years after treatment in 57.1% of cured and 100% of non-cured patients. Immunoblotting, based on AgB subunits (8 and 16 kDa), revealed 14.3% of seropositivity after 4 years, with no reactivity to the components after 5 years of follow up. Interestingly, WB remained positive in 100% of non-cured patients up to 5 years (end of follow-up period). Serum antibodies to a certain bands (24 and 39) of HCF in Western blotting decreased in post-surgical monitoring of CE patients[70].

CONCLUSION

The performances of currently available immunodiagnostic test in diagnosis of CE are not satisfactory and the best serological test for diagnosis of CE is still the subject of debate. Over the time, particularly during the last two decades, several immunodiagnostic tests have been developed, mainly based on HCF, AgB and Ag5, yet their performance in diagnosis of human hydatidosis are unsatisfactory.

The most widely used antigens for serological diagnosis of CE are AgB and Ag5. Yet new antigens are being constantly evaluated and new serological assays are being developed to improve the performance of serological diagnostic tests.

Utilizing of recombinant or synthetic antigen although improved the performance, but has not overcome the problem of low sensitivity or even cross reactivity with other antigen in diagnosis of CE and these problems still remained. Considerable variation in performance of serological test for diagnosis of CE between different laboratories is mainly related to lack of standardization of antigen preparation, inadequate sensitivities and specificity, and also strain of the parasite that antigens have been purified from its content.

Immunodiagnostic tests based on recombinant antigen has drawn the attention of many researchers and the outcomes of such studies are promising. These antigens, especially based on AgB subunits, showed not all the times, but in most cases, satisfactory performance in comparison to their homologues antigens.

New interesting perspective in the development of serological assays for diagnosis of CE might be derive from recent observation that IgG subclasses are good markers for diagnosis and also follow up of CE patients. Moreover, the evaluation of highly purified Ag5 for immunodiagnosis of CE seems to be a promising task ahead which must be undertaken in the future. And finally immunodiagnosis assays may well be improved through combining of several well-defined antigens, notably immunodominant antigen in different stages of the cyst development.

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