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Rapid Diagnostic Tests for the Serodiagnosis of Human Cystic Echinococcosis

Tests de diagnostic rapide pour le sérodiagnostic de l'échinococcose kystique humaine

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Abstract Cystic echinococcosis (CE) is a parasitic zoonosis especially affecting resource-poor populations in livestock raising areas. Imaging, in particular ultrasound (US), is crucial for the diagnosis, staging, and clinical management of abdominal CE in humans. Serology is a valuable complement to imaging, especially when ultrasound features of CE are absent or unclear. In rural endemic areas, where expertise in US is scant, and conventional serology techniques are unavailable due to lack of laboratory equipment, rapid diagnostic tests (RDTs) may be very useful. Several reports have described the performance of commercial and experimental RDTs in the diagnosis of CE, including a recent study by our group that compared the diagnostic performances of three commercial RDTs for the diagnosis of hepatic CE. To put RDTs for CE in context, we reviewed the available literature in English on this topic. Overall, RDTs appear to be useful in resource-poor settings where they may replace conventional serodiagnostic tests. However, like other serodiagnostic tests, RDTs lack standardization and show unsatisfactory sensitivity and specificity. An important issue that needs to be addressed is that studies on the diagnostic performance of RDTs fail to take into account the variables known to influence results such as anatomical location and cyst stage.

Keywords Rapid diagnostic test · RDT · Cystic echinococcosis · Hydatidosis · Serodiagnosis · Diagnostic accuracy

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Résumé L'échinococcose kystique (EK) est une zoonose due à un parasite touchant particulièrement les populations à ressources limitées dans les zones d'élevage du bétail. L'imagerie, notamment l'échographie, est cruciale pour le diagnostic, la détermination du stade et la prise en charge clinique de l'EK abdominale chez l'homme. La sérologie est un complément intéressant à l'imagerie, en particulier quand les caractéristiques échographiques de l'EK sont absentes ou peu claires. Dans les zones endémiques rurales, où la maîtrise de l'échographie est faible et où les techniques conventionnelles de sérologie ne sont pas disponibles du fait du manque d'équipements de laboratoire, les tests de diagnostic rapide (TDR) peuvent être très utiles. Plusieurs études ont décrit les performances des TDR expérimentaux ou du commerce dans le diagnostic de l'EK, dont une étude récente menée par notre groupe qui compare les performances de trois TDR du commerce pour le diagnostic de l'EK hépatique. Pour replacer les TDR de l'EK dans leur contexte, nous avons effectué une revue de la littérature en anglais sur ce thème. Généralement, les TDR se sont montrés utiles dans les établissements à faibles ressources, où ils peuvent remplacer les tests conventionnels de sérodiagnostic. Cependant, comme pour les autres tests de sérodiagnostics, les TDR ne sont pas standardisés, et leur sensibilité et spécificité ne sont pas satisfaisantes. Une question importante qui mérite d'être soulevée est que cette étude sur les performances de diagnostic des TDR ne prend pas en compte les variables connues pour avoir une influence sur les résultats, comme la localisation anatomique et le stade du kyste.

Mots clés Test de diagnostic rapide · TDR · Échinococcose kystique · Hydatidose · Sérodiagnostic · Précision du diagnostic

Introduction

Cystic echinococcosis (CE) is a parasitic zoonosis caused by the metacestode (larval stage) of the dog tapeworm

Echinococcus granulosus sensu lato species complex. The life cycle of the parasite develops between canids (definitive hosts), and livestock, particularly sheep (intermediate hosts). The infection is prevalent worldwide especially in rural livestock-raising areas such as the Mediterranean, Eastern Europe, North and East Africa, South America, Central Asia, and China. Recent estimates indicate that 1.2 million people are affected worldwide and 3.6 million Disability Adjusted Life Years (DALYs) are lost due to CE [7]. Humans are dead-end intermediate hosts, where fluid-filled cysts (hydatid cysts) develop mostly in the liver, after ingestion of infective eggs passed with dog feces contaminating the environment.

Most cases remain asymptomatic or paucisymptomatic for a long time or even indefinitely, and may be diagnosed accidentally; the spectrum of clinical manifestations ranges from asymptomatic to extremely serious conditions (e.g., anaphylactic shock). The mainstay of diagnosis and staging of CE is imaging, in particular ultrasound (US) for abdominal localization of CE cysts; US-based imaging guides the clinical management of infected patients [4,20]. In specific circumstances, such as when US is not available, in case of extra-abdominal CE, complications (e.g., to evaluate cystobiliary fistulas), and during pre-surgical evaluation, T2-weighted MRI (Magnetic Resonance Imaging) is preferable as CT (Computed Tomography) does not satisfactorily reproduce specific features of CE on which diagnosis and clinical management decisions are based [4,20].

Cysts are categorized into six US stages according to the WHO Informal Working Group on Echinococcosis (WHO-IWGE) classification [4,26]. Based on this classification, a stage-specific management approach is recommended [4]. However, specific expertise in US and on the recognition of the pathognomonic features of CE cysts on imaging are needed for a correct diagnosis and management of the infection to avoid unnecessary or inappropriate treatment, with the consequent risks and costs. The differential diagnosis of CE is broad, ranging from harmless biliary cysts to cancer, and this poses serious problems in endemic, resource-poor settings where expertise in US and availability of invasive options for differential diagnosis are scant.

Serology is a valuable complementary tool when imaging features are unclear, although currently available tests lack standardization and present unsatisfactory sensitivity and specificity [5,14]. In underserved rural endemic areas, conventional serology techniques are often unavailable or unreliable due to the lack of laboratory equipment and expertise. In this context, the use of rapid diagnostic tests (RDTs) may be useful to complement imaging when needed. Several reports have described the performance of commercial and experimental RDTs in the diagnosis of CE, including our own comparing the diagnostic performances of three commercial RDTs for hepatic CE. Here, we present a review of the published literature on this topic.

Methods

On February 14, 2016, we performed a PubMed (MEDLINE) literature search using the search: ((echinococcus) OR (echinococcus granulosus) OR (cystic echinococcosis) OR (hydatid disease) OR (hydatidosis)) AND (((serodiagnosis) OR (serodiagnostic test) OR (rapid diagnostic test) OR (immunochromatograph*) OR (dot immuno*) OR (immunofiltration))).

Original papers reporting data from individual patients with cystic echinococcosis (diagnosed surgically or by imaging) were considered eligible if they described the performances of experimental or commercial serodiagnostic tests broadly fulfilling the definition of the WHO for simple/rapid tests (www.who.int/diagnostics_laboratory/faq/simple_rapid_tests, accessed on 26/01/2016): (i) High quality, easy-to-use tests for use in resource-poor settings; (ii) based on agglutination, immuno-dot, immunochromatographic and/or immuno-filtration techniques; (iii) quick—10 min to 2 h—and easy to perform and requiring little or no additional equipment; (iv) designed for use with individual or a limited number of samples, which make them more economical than ELISAs in low-throughput laboratories. The capacity to be stored at room temperature for an extended period of time, included in the WHO definition, was not an inclusion criterion in our search. Hemagglutination tests were excluded from our search because they do not comply with the typical features of a point-of-care test. Other test formats (e.g., dot-ELISA, latex agglutination tests, etc.) were included in the analysis only if the described methods were compatible with the features of a point-of-care test described earlier. Exclusion criteria were: (i) review papers; (ii) manuscripts where the methods were not described and therefore the applicability of the definition for rapid test was not assessable; (iii) publications in languages other than English without available abstract in English. When the full text was not available, the abstract in English was used for the required information and included if the inclusion criteria were met.

Data extracted from the selected publications included sensitivity, specificity, test comparator, reference standard, information on characteristics of the samples (number, report of collection before or after treatment, type of control samples), and information on characteristics of the cysts (report of cyst localization, stage and number).

Results

The PubMed search returned 1044 results (Fig. 1). Of these, 16 publications met the inclusion criteria. Full text was

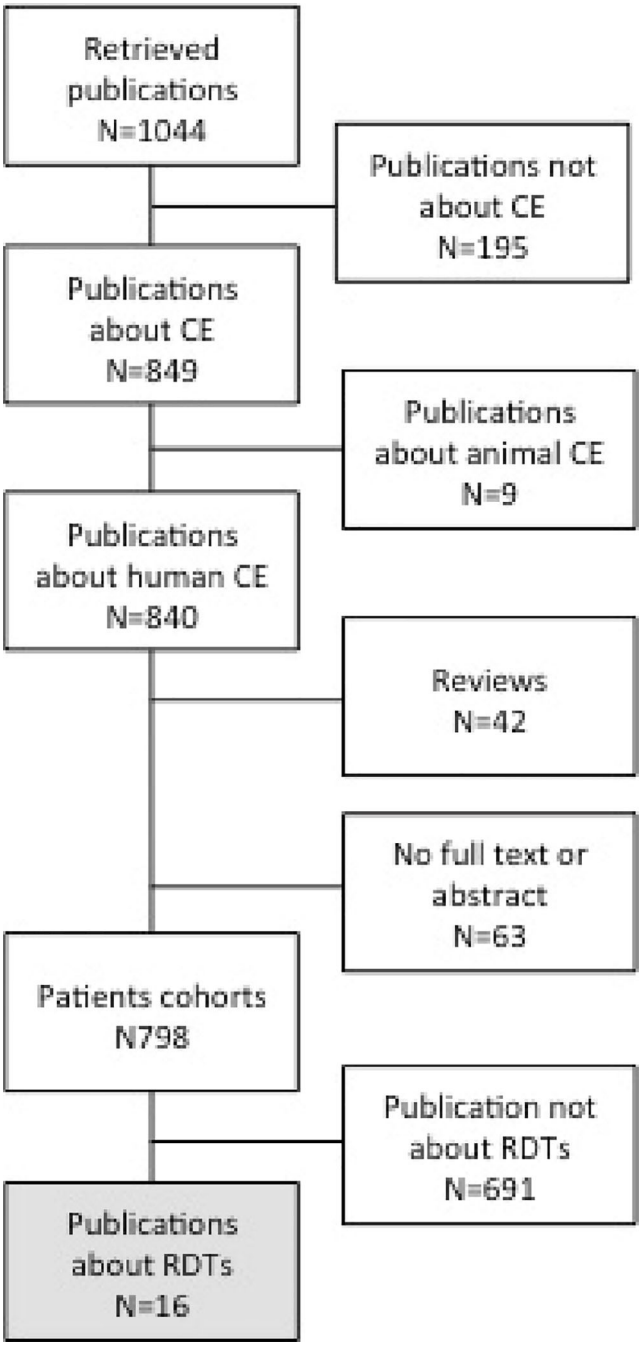

Fig. 1 Flowchart of the PubMed search and selection of eligible publications. Eligible publications are indicated by gray boxes / *Organigramme de la recherche PubMed : sélection des publications éligibles. Les publications éligibles sont dans la case grisée*

available for 15 out of 16 publications. Five publications described the performances of commercialized RDTs; however, one of the tests described was out of production at the time of writing.

The characteristics of the assays described in the 16 included works are summarized in Table 1, while Table 2 summarizes the tests performance and characteristics of the studied population used for their assessment. With the exception of the publication by Xie et al. [27] from which the information was not provided in the available abstract, all other works assessed the performances of RTDs in case-control studies, while Feng et al. [8] and Rogan et al. [17] also evaluated the tests in the field.

Discussion

In rural areas where CE is endemic, and health care facilities are basic and/or difficult to access, RDTs may be useful in the differential diagnosis of lesions compatible with CE on imaging.

Different rapid test formats, based on different antigens, have been assessed over time for the diagnosis of CE, using mostly sera from patients with surgically confirmed CE infection (Tables 1, 2). Slide latex agglutination tests were the most commonly used rapid test types until the 1990s, while more recent studies mostly evaluated immunochromatographic assays, a format that better responds to the characteristics of a point-of care test. Although it is impossible to compare the performances of different assays on the sole basis of the published literature, overall, RDTs showed performances comparable with those of conventional serology techniques, both in overall, organ-specific, and stage-specific sensitivity and specificity values. However, the variables associated with serology results [9,13], and fundamental in the interpretation and evaluation of serodiagnostic test results, were not always taken into consideration (Table 2).

Four recent studies evaluated the performances of the three currently commercially available RDTs for the diagnosis of CE and employable in both hospital and field settings (VIRapid HYDATIDOSIS [Vircell, Spain], Echinococcus DIGFA [Unibiotest, China], and ADAMU-CE [ICST, Japan]) [8,18,22,23]. The performances of these three tests in the diagnosis of hepatic CE were compared in the recent work of Tamarozzi et al. [22]. In this work, using US imaging with WHO-IWGE classification as the gold standard and RIDASCREEN Echinococcus IgG ELISA (R-Biopharm, Germany) as the test comparator, the VIRapid HYDATIDOSIS test showed the best diagnostic accuracy (overall Se 74.1% and Sp 96%; Se 89.2% on samples from patients with active CE cysts) among the RDTs, statistically comparable with that of the ELISA test. The ADAMU-CE test was significantly less sensitive in the diagnosis of CE in patients with active cysts (Se 71.1% on samples from patients with active CE cysts), and the DIGFA test was significantly less specific (Sp 72%). These results are in accordance with the literature, showing that tests based on recombinant antigens have generally better sensitivity, while those based on native antigens have generally better specificity [5,9,14]. Among the publications included in this review, Chen and colleagues [6]

Table 1 Characteristics of the rapid diagnostic tests / *Caractéristiques des tests de diagnostic rapide*

Publication	RDT format	Antigen used	Production
Tamarozzi et al. (2016) [22]	ICT	AgB/Ag5	Commercial: VIRapid HYDATIDOSIS (Vircell, Spain)
	ICT	rAgB	Commercial: ADAMU-CE (ICST, Japan)
	DIGFA	HCF/Psc/AgB/Em2	Commercial: Echinococcus DIFGA (Unibiotest, China)
Xie et al. (2015) [in Chinese] [27]*	ICT (read with a reader)	N/S	In house
Chen et al. (2015) [6]	DIGFA	rAgB	In house
Santivanez et al. (2015) [18]	ICT	rAgB	Commercial: ADAMU-CE (ICST, Japan)–different version from test used by Tamarozzi et al.
Tamer et al. Med Sci Monit (2015) [23]	ICT	AgB/Ag5	Commercial: VIRapid HYDATIDOSIS (Vircell, Spain)
Wang et al. Parasitol Res (2013) [25]	ICT	HCF	In house
Feng et al. Acta Trop (2010) [8]	DIGFA	HCF/Psc/AgB/Em2	Commercial: Echinococcus DIFGA (Unibiotest, China)
Olut et al. (2005) [15]	Dot-immunobinding	HCF	Echinostrip, developed by Lofarma Laboratories, Italy
Al-Sherbiny et al. (2004) [1]	Dipstick	HCF	In house
Babba et al. (2004) [2]	Slide-LAT[a]	HCF	In house
Barbieri et al. (1993) [3]	Slide-LAT	HCF lipoprotein fraction	In house
Rogan et al. (1991) [17]	Rapid dot-ELISA	AgB	In house
Hoghooghi et al. (1976) [11]	Slide-LAT	N/S	Commercial: Italdiagnostico Co, Italy (no more on the market)
Varela-Diaz et al. (1975) [24]	Slide-LAT[a]	HCF	In house
Kagan et al. (1966) [12]	Slide-LAT[a]	HCF	In house
Szyfres and Kagan (1963) [21]	Slide-LAT	HCF	In house

Abbreviations:RDT: Rapid Diagnostic Test; ICT: Immunochromatographic Test; DIGFA: Dot Immunogold Filtration Assay; Slide-LAT: Slide Latex Agglutination Test; rAgB: recombinant Antigen B; Ag5: Antigen 5; HCF: Hydatid Cyst Fluid; Psc: Protoscolex antigen; Em2: Echinococcus multilocularis Em2 antigen ; N/S: Not Specified; ELISA: Enzyme Linked Immunosorbent Assay
* Only abstract available in English. [a] Slide-LAT based on the method of Szyfres and Kogan (1963)
Publications are listed in reverse order of publication date

reported that the use of recombinant antigens in an in house version of the DIGFA test might improve the specificity of the test, but at the expense of sensitivity. It must be pointed out that the approach of Tamarozzi and colleagues in the evaluation of RDTs was clinically oriented, with test specificity assessed using samples from patients with lesions on imaging posing differential diagnosis problems with CE. A thorough evaluation of tests specificities using a panel of sera from patients with a wide set of other potentially cross-reacting helminthic infections and other non-infectious diseases would be a necessary further step to assess the potential usefulness of these assays in the field, in countries where helminth multiparasitism is often common and a proportion of false-positive results in supposed healthy individuals could be due to infection with other parasites. However, data on concomitant infections is often difficult to obtain for several reasons, and studies based on serology alone, without previous visualization of compatible lesions by imaging, should be performed and interpreted with extreme caution.

The importance of taking into account cyst-related clinical variables known to influence the results of serodiagnostic test is exemplified by the results obtained when other groups evaluated the same commercial tests.

Tamer and colleagues [23] recently evaluated the performances of the VIRapid test and reported a better Se (96.8%) and same Sp (96% after excluding sera from patients with other parasitic conditions) compared with the results of Tamarozzi et al. [22]. However, they did not provide data

Table 2 Test performances and characteristics of the study / *Performances et caractéristiques des tests de l'étude*

Ref	RDT format	Reported RDT Se and Sp	*N* samples from patients with CE tested	CE diagnosis gold standard	Comparator test format and performances	Sampling time point	Cysts characteristics considered in the analysis	Samples from control subjects used for Sp calculations
[22]	ICT: VIRapid HYDATIDOSIS (Vircell, Spain) ICT: ADAMU-CE (ICST, Japan) DIGFA: Echinococcus DIFGA (Unibiotest, China)	Se 74.1% Sp 96% Se 57.6% Sp 100% Se 72.9% Sp 72%	59	Ultrasound	Commercial ELISA: RIDASCREEN Echinococcus IgG (R-Biopharm, Germany). Se 69% Sp 96%	Before treatment or > 12 m after treatment	Yes (stage, number [1 cyst], and localization [only hepatic cysts])	Patients with non-parasitic liver cysts
[27]*	ICT (read with a reader)	Se 95.6% Sp 93.7%	159	N/S	ICT (read by operator) Se 93.7% Sp 85%	N/S	N/S	Patients with other liver diseases (N/S)
[6]	DIGFA	Se 77.9% Sp 91.9% [83.2% if samples from patients with AE included]	113	Surgery	DIGFA based on native AgB Se 92.9% Sp 84.1% [71% if samples from patients with AE included]	N/S	N/S	Patients with AE, other parasitoses, other liver diseases, and healthy individuals
[18]	ICT ADAMU-CE (ICST, Japan) – different version from test used by Tamarozzi et al.	Se 80% on samples from patients with liver cysts; 76% with lung cysts Sp 100% [89.8% if samples from patients with AE included]	50	Surgery	In house ELISA Se 76% on samples from patients with liver cysts; 68% with lung cysts Sp N/S	N/S	Yes (number and localization)	Patients with AE, other parasitoses, and healthy individuals

(Suite page suivante)

Table 2 *(suite)*

Ref	RDT format	Reported RDT Se and Sp	*N* samples from patients with CE tested	CE diagnosis gold standard	Comparator test format and performances	Sampling time point	Cysts characteristics considered in the analysis	Samples from control subjects used for Sp calculations
[23]	ICT	Se 96.8% Sp 87.5%	102	Surgery	Commercial ELISA: Echinococcus IgG (DRG, Germany) Se 98.9% Sp 70%	Before treatment	N/S	Patients with other parasitoses (AE not included), and healthy individuals
[25]	ICT	Se 91% Sp 95.7% [78% if samples from patients with AE included]	144	Surgery or imaging	In house ELISA Se 92.4% Sp 92.2% [72.8% if samples from patients with AE included]	Before treatment	Yes (stage)	Samples from patients with AE, other parasitoses, non-parasitic liver cysts, and healthy individuals
[8]	DIGFA: Echinococcus DIFGA (Unibiotest, China)	Se 80.7% hospital samples [83.4% on samples from patients with liver cysts; 80.7% with lung cysts]; 71.8% samples from field study Sp 97% [93.4% if samples from patients with AE included] hospital samples; 78.1% samples from field study [N/S with/without samples from patients with AE]	857 (hospital samples) 160 (samples from field study)	Surgery (hospital) Ultrasound (field study)	In house ELISA Se 75% hospital samples; N/S samples from field study Sp 96% [93.4% if samples from patients with AE included] hospital samples; N/S samples from field study	After treatment (hospital samples) Before treatment (samples from field study)	Yes (localization [abdominal and extra-abdominal CE for hospital samples; only abdominal CE for samples from field study]; stage)	Hospital samples: from patients with AE, cysticercosis, other infectious and non-infectious conditions, and healthy individuals Samples from field study: from patients with AE and healthy individuals

(Suite page suivante)

Table 2 *(suite)*

Ref	RDT format	Reported RDT Se and Sp	*N* samples from patients with CE tested	CE diagnosis gold standard	Comparator test format and performances	Sampling time point	Cysts characteristics considered in the analysis	Samples from control subjects used for Sp calculations
[15]	Dot-immuno-binding	Se 67% Sp 100%	18	Surgery	Commercial IHA: Cellognost-Echinococcus IHA (Behring, Germany) Se 50% Sp100%	Either before or after treatment	Yes (localization [only lung cysts])	Samples from patients with teniasis, other infectious and non-infectious diseases, and healthy individuals
[1]	Dipstick	Se 100% Sp 91.4%[N/S with/ without samples from patients with AE samples]	26	Surgery	In house EITB Se 100% Sp 91.4% In house ELISA Se 91.6% Sp 100%	N/S	N/S	Samples from patients with AE, other parasitoses, and healthy individuals
[2]	Slide-LAT[a]	Se 64.9% on samples from patients with liver cysts; 64.7% with lung cysts Sp N/S	243	Surgery	In house ELISA Se 89.2% on samples from patients with liver cysts; 77.9% with lung cysts Sp N/S In house CIE Se 82.9% on samples from patients with liver cysts; 75.6% with lung cysts Sp 100%	N/S	Yes (localization; not clear what test used for stage correlation)	Samples from patients with other infectious and non-infectious diseases
[3]	Slide-LAT	Se 87% [97.5% on samples from patients with liver cysts; 91.6% with lung cysts] Sp 92.3% [87.9% if samples from patients with AE samples included]	119	Surgery	In house ELISA Se 83% [98% on samples from patients with liver cysts; 88% with lung cysts] Sp 94.5% [87.8% if samples from patients with AE included]	N/S	Yes (localization)	Samples from patients with AE, other parasitoses, other infectious and non-infectious diseases, and healthy individuals

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Table 2 *(suite)*

Ref	RDT format	Reported RDT Se and Sp	*N* samples from patients with CE tested	CE diagnosis gold standard	Comparator test format and performances	Sampling time point	Cysts characteristics considered in the analysis	Samples from control subjects used for Sp calculations
[17]	Rapid dot-ELISA	Se 94% both hospital samples and samples from field study Sp 93.3% hospital samples [77.8% if samples from patients with AE included]; 94.4% samples from field study	100 (hospital samples) 17 (samples from field study)	Clinical diagnosis (hospital samples; techniques N/S) Ultrasound (field study)	In house ELISA Se N/S Sp N/S	N/S	N/S	Hospital samples: from patients with AE, other parasitoses, bacterial infections, and healthy individuals Samples from field study: from ultrasound-negative subjects
[11]	Slide-LAT: Italdiagnostico Co, Italy (no longer in the market)	Se 83.3% on samples from patients with liver cysts; 82.3% with lung cysts; 80% with cysts in other sites Sp 95.2%	51	Surgery	Casoni reaction skin test Se 79.2% on samples from patients with liver cysts; 76.4% with lung cysts Sp N/S	N/S	Yes (localization)	N/S
[24]	Slide-LAT[a]	Se 50% Sp N/S	30	Surgery	CIE Se 50% Sp N/S	Before treatment	N/S	Samples from patients with other parasitoses, non-parasitic diseases, and healthy individuals
[12]	Slide-LAT[a]	Se 82% on samples from patients with liver cysts; 44% with lung cysts Sp 92%	57	Surgery	In house IHA Se 82% on samples from patients with liver cysts; 33% with lung cysts	N/S	Yes (localization)	Samples from patients with teniasis, other non-parasitic diseases, and healthy individuals

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Table 2 *(suite)*

Ref	RDT format	Reported RDT Se and Sp	*N* samples from patients with CE tested	CE diagnosis gold standard	Comparator test format and performances	Sampling time point	Cysts characteristics considered in the analysis	Samples from control subjects used for Sp calculations
[21]	Slide-LAT	Se 100% Sp 97%	23	Surgery	In house IHA Se N/S Sp N/S	N/S	N/S	Samples from patients with other parasitoses, and non-parasitic infections

* Only abstract available in English. [a]. Slide-LAT based on the method of Szyfres and Kogan (1963)

Abbreviations: RDT: Rapid Diagnostic Test; Se: Sensitivity; Sp: Specificity; AE: Alveolar Echinococcosis; N CE: Number of samples from patients with CE; ICT: Immunochromatographic Test; DIGFA: Dot Immunogold Filtration Assay; AgB: Antigen B; Slide-LAT: Slide Latex Agglutination Test; IHA: Indirect Hemagglutination Assay; EITB: Electroimmunotransfer Blot; CIE: Counterimmunoelectrophoresis; ELISA: Enzyme Linked Immunosorbent Assay; N/S: Not Specified

Publications are listed in reverse order of publication date

on cyst characteristics. In their work, all sera from CE infected patients were from surgically confirmed cases, suggesting that predominantly sera from patients with active CE stages (more likely to be seropositive [13]) may have been included in their cohort. When excluding healthy subjects, Sp was 85.3%, and the impact of other helminthic infections, which could be suspected due to the 4% false-positive rate in healthy donors, remained to be evaluated.

Feng and colleagues [8] evaluated commercial DIGFA and reported a sensitivity of 83.4% on sera from patients with hepatic CE and a specificity of 93.4% when sera came from hospitalized patients. When evaluated by Tamarozzi et al. [22], the DIGFA test gave clearly inferior results. However, the test performances were comparable with those found by Feng and colleagues [8] when sera from US screening campaigns were used (Se 71.8% on sera from patients with abdominal CE; Sp 78.1%). The different performances of the tests when using samples from the two settings were attributed by the authors to the presence, in the field setting, of subjects exposed to the parasite without developing detectable infection, or of seronegative subjects with old lesions. However, other variables that may account for these discrepancies are (i) time of serum collection relative to treatment (sera from hospitalized patients were collected < 2 years after surgical treatment for CE, and it is known that seroconversion and increase in antibody titers occur after therapy [13]; (ii) different distribution of CE stages in the two patient cohorts (not detailed in the publication), with probably more sera from patients with active cysts in the hospital compared with the field survey cohort; and (iii) different prevalence of cross-reacting helminth co-infections in the two populations.

The ADAMU-CE test in a previous "version" than that used in the work of Tamarozzi et al. [22], was evaluated by Santivanez and colleagues [18] on a panel of sera from patients with surgically confirmed CE and from patients with a wide set of other helminth infections. Unfortunately, sera from patients with possibly interfering non-infectious conditions were not tested. The authors reported a better Se (80% on sera from patients with liver CE cysts) and same Sp (100% if sera from patients with alveolar echinococcosis or sera from healthy controls were excluded) compared with the results of Tamarozzi et al. The differences in the results of the two studies may be at least in part due to the different distribution of CE stages in the two cohorts (not detailed in the work of Santivanez et al.) and to differences in the test characteristics between the two "versions" of the kit.

In line with the literature [13,16,28], all RDTs were as poorly sensitive as the comparator tests in the diagnosis of CE in stage CE1 and inactive stages, when cyst stage was taken into consideration in the analysis of results. This confirms the limits of serology in the diagnosis of CE and in supporting the differential diagnosis of CE1 and CE4–CE5

cysts from other hepatic lesions. Indeed, young CE1 cysts and inactive CE4–CE5 cysts may not show pathognomonic signs and, unfortunately, these stages are also those with the broader differential diagnosis (e.g., simple cysts, neoplastic lesions). In these cases, therefore, other diagnostic strategies are needed, including imaging (contrast-enhanced CT or MRI), empiric treatment with albendazole (seronegative patients with active CE generally seroconvert in a variable time after treatment), and diagnostic puncture/biopsy. The percentage of positive results according to cyst stage obtained with a commercial RDT is schematized in Figure 2. Also in line with the literature [9], all RDTs were as poorly sensitive as the comparator tests in the diagnosis of CE in extra-hepatic localization, when cyst localization was taken into consideration in the analysis of results. Finally, the operator dependence of RDTs reading, especially in the presence of a faint signal, is a known issue [10,19]. The results of Xie and colleagues [27] suggest that the use of an automatic reader may improve the diagnostic accuracy of the test. However, the cost-effectiveness of such approach needs evaluation.

To conclude, it appears that RDTs have overall comparable performance to the routine serodiagnostic tests in the diagnosis of CE, supporting their possible use in resource-poor settings to complement imaging diagnosis of CE in doubtful cases. However, significant differences in diagnostic accuracy exist among them and the heterogeneity of the antigens used, of the studies design and of the description of samples characteristics makes it impossible to compare the performances of different assays on the sole basis of published literature. When cyst stage was taken into account, RDTs, similar to conventional tests, showed poor sensitivity in the presence of inactive CE4–CE5 and of CE1 cysts, which are cyst stages that may pose considerable problems of differential diagnosis, and in the diagnosis of extra-hepatic CE.

Further studies are warranted to evaluate the use of whole blood from finger prick sampling, and certainly to evaluate any advantage of RDTs, and serological tests in general, in the diagnosis and clinical management of CE, which is still to be proven. Once again, a crucial point is that the calibration and evaluation of diagnostic tests should be performed first on well-characterized and homogeneous samples, and subsequently in "real life" settings. Instead, this is rarely done, with the attending problems in the interpretation and meaningfulness of the results. Furthermore, case-control studies tend to overestimate test accuracy; therefore, further studies should assess the performances of RDTs through cohort studies in relevant populations. Equally important is the choice of control samples. Non-parasitic cysts (e.g., simple cysts, neoplasms) represent the most common differential diagnosis of hepatic CE cysts, and samples from patients with such conditions need to be tested. Samples from patients with alveolar echinococcosis should also be evaluated to assess the specificity of the test. Indeed, *E. multilocularis* is the main cross-reacting infection for CE immunoassays, and lesions caused by *E. multilocularis* may pose frequent problems in differential diagnosis with CE, with serious, life-threatening consequences for the patient; this is of particular concern in co-endemic areas. Also, it is important to assess cross-reactivity in the presence of other helminthic infections and the inclusion of samples from patients with non-parasitic conditions potentially interfering with test performances (e.g., hypergammaglobulinemia, autoantibodies, other infections such as syphilis and hepatitis). The difficulty in accessing such a vast spectrum of control samples results in unsatisfactory knowledge of the real specificity of immunoassays, which is overestimated by the inclusion of samples from supposed healthy subjects in Sp calculations. However, it must be stressed that serology for CE should be performed only after lesions compatible with echinococcosis are found by imaging, both in the context of

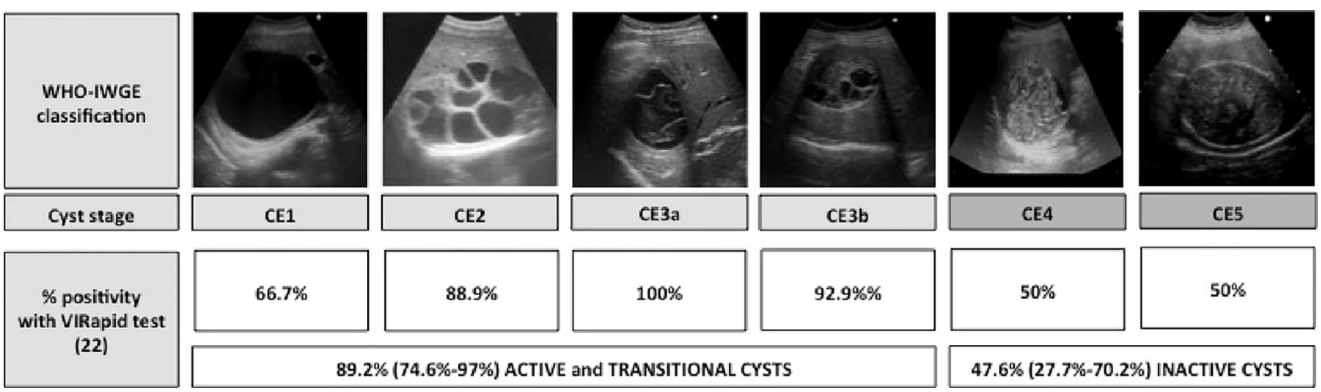

Fig. 2 Percentage of positive results according to CE cyst stage obtained with the commercial RDT VIRapid HYDATIDOSIS (Vircell, Spain) [22] / *Pourcentage de résultats positifs en fonction des stades des kystes d'EK obtenus avec les TDR du commerce VIRapid HYDATIDOSIS (Vircell, Espagne)* [22]

field population studies and in the clinical setting. This will increase the pre-test probability of the presence of infection, otherwise very low. Factors concurring to the low predictive value of serology results in the absence of compatible lesions are: (i) the low prevalence of infection (generally < 10%) even in highly endemic areas; (ii) the generally low specificity of serodiagnostic tests (especially an issue in areas where exposure to the parasite without subsequent establishment of infection may occur and where other cross-reacting infections may be prevalent and often not investigated/investigable in the context of population studies); and (iii) the low sensitivity of serodiagnosis in case of extra-hepatic CE (limiting the use of serology to diagnose extra-hepatic CE).

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