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Large-scale evaluation of a rapid diagnostic test for human cystic echinococcosis

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ABSTRACT

Cystic echinococcosis (CE) is a neglected zoonotic disease, diagnosed through clinical findings, imaging techniques, and serology, for which many serological tests are available. Here we report a rapid unit assay, the immunochromatographic VIRapid® HYDATIDOSIS test (Vircell, Granada, Spain), potentially suitable for laboratories in low-prevalence or poorly equipped areas. This test was evaluated with a large retrospective cohort (224 sera), including patients suffering from CE or from other parasitic or liver diseases. The test was also assessed in routine conditions with a prospective cohort (115 sera) in areas where both cystic and alveolar echinococcoses have been diagnosed. Its performance (in terms of sensitivity, specificity, and both positive and negative likelihood ratios) was similar to an ELISA based on a crude antigen. Our study shows that this test performs adequately in the diagnostic process, when used with caution, especially regarding cross-reactivity with other parasitic diseases.

1. Introduction

Human echinococcoses are caused by the larval stage of parasites belonging to the genus *Echinococcus*. The main forms are cystic echinococcosis (CE, also called hydatidosis) due to *E. granulosus* sensu lato, and alveolar echinococcosis (AE) due to *E. multilocularis*. The definitive hosts of the parasite are carnivores, principally dogs for CE, and foxes for AE. Humans are infected through ingestion of parasite eggs. Living in rural endemic areas with the presence of free-roaming dogs (for CE) and/or foxes (for AE), and being a dog owner are among the parameters that lead to a higher risk of human infection (Deplazes et al., 2017; Piarroux et al., 2013; Possenti et al., 2016).

Echinococcoses are chronic, complex and neglected diseases that mainly affect the liver and/or lungs. They represent a public health problem, affecting more than one million people (World Health Organization and World Organisation for Animal Health, 2011). *Echinococcus granulosus* sensu lato has a worldwide distribution, with the highest prevalence of CE in the Mediterranean region, China and South America. *Echinococcus multilocularis* is limited to the northern hemisphere, with central Europe, Turkey, Russia, and China being the main regions affected (Deplazes et al., 2017). In several vast regions of the world, such as Turkey, Russia and China, the 2 species are co-endemic, thus making differential diagnosis more difficult.

Both cystic and alveolar echinococcoses are complicated to treat, sometimes requiring extensive surgery and/or prolonged drug therapy (Brunetti et al., 2010; Nunnari et al., 2012). In both CE and AE, infection is followed by a long asymptomatic incubation period, which can last several years before diagnosis and/or occurrence of symptoms. The *E. multilocularis* metacestode develops as a slowly growing, infiltrative, tumor-like mass lesion, with a primary lesion able to spread by infiltration and metastasis. When symptomatic, patients generally present with icterus, abdominal pain and/or hepatomegaly. Development of *E. granulosus* sensu lato larvae leads to single, or sometimes multiple, well-delimited fluid-filled cysts. Secondary cysts in other organs or tissues result from spontaneous or trauma-induced primary cyst rupture. Clinical symptoms usually occur when the cyst compresses or ruptures into neighboring structures. Because of the potentially long clinical latency, diagnosis may be fortuitous, during examination by ultrasound and/or other imaging techniques, or can occur at a later stage, when symptoms are present. Early diagnosis and prompt treatment are essential to treat these diseases efficiently.

Diagnosis is based on clinical findings, imaging techniques (ultrasonography is typically used; conventional radiography, computed axial tomography and/or magnetic resonance imaging may also be required in certain cases), and serology (Brunetti et al., 2010). PCR-based

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methods are also available for the molecular detection of the parasite and can be very helpful (Grenouillet et al., 2013; Siles-Lucas et al., 2017). Depending on the prevalence of the disease and the resources available in a given region, the radiologist may be more or less experienced in recognizing echinococcosis lesions (Bartholomot et al., 2002; Romig et al., 1999), while laboratories in the region may be more or less well equipped to perform serological analysis. For the 2 diagnoses, many different suppliers produce serological tests, belonging to 3 different categories: ELISA, indirect hemagglutination, and western blot. The performance (sensitivity and specificity) of these tests depends on the antigen used (crude antigen or recombinant protein) (Carmena et al., 2006, 2007), the disease stage, the site of the lesion, the number and size of lesion(s), and any anthleminthic treatments (Lissandrin et al., 2016; Manzano-Román et al., 2015; Wang et al., 2013). The characteristics of the lesions have a higher impact on diagnosis performance for CE. Without clear clinical images and/or the possibility of confirming the diagnosis with serology, echinococcosis can be underdiagnosed or misdiagnosed, leading to inadequate patient management (Stojkovic et al., 2015). In the differential diagnosis of echinococcosis, a misdiagnosis, especially of neoplasm or of CE in AE patients, has serious consequences for the patient.

Developing accurate and sensitive rapid diagnostic tests (RDT) based on immunochromatography could solve these problems of laboratory and/or equipment availability (notably for field use), by facilitating rapid individual testing in low-prevalence areas (where laboratories have few serology requests), and speedy confirmation of results from another technique. In this way, the immunochromatographic test VIRapid® HYDATIDOSIS (Vircell, Granada, Spain) has been developed, based on an HPLC-purified *E. granulosus* 5/B enriched antigen. This test is a rapid-to-apply solution with easy-to-read results. The supplier advertises excellent performance, with 94.7% sensitivity and 99.5% specificity, but only blood donors and highly immunoreactive CE patients were tested (Delgado et al., 2010). Two previous studies have described the performance of this RDT in different contexts (Tamarozzi et al., 2016; Tamer et al., 2015). In Tamarozzi et al., which tested 190 sera, the control group was patients with non-parasitic hepatic cysts, and in Tamer et al., which tested 84 sera, the control group was healthy donors and patients with other parasitic diseases. However, no previous study has included patients with alveolar echinococcosis, nor prospectively assessed this test.

The aim of our study is therefore to evaluate the performance of the RDT from Vircell, with a large panel of patients, including a retrospective panel of well-defined sera, and a prospective cohort representative of Echinococcus serologies, routinely performed in a laboratory for a variety of reasons in an endemic area of AE, with a lower frequency of CE infection.

2. Material and methods

2.1. Clinical specimens

This study included 2 cohorts of patients: a retrospective panel of sera from patients with well-defined diagnoses, and a prospective serum panel. All sera were collected at diagnosis, thus excluding sera for serological follow-up of patients.

2.1.1. Retrospective cohort

A total of 224 serum samples was included:

- 94 sera from patients with confirmed (n = 66) or probable CE (n = 28), and 25 sera from patients with confirmed or probable AE, according to WHO criteria (Brunetti et al., 2010). Clinical and epidemiological characteristics of CE patients are given in the supplementary data file;
- 43 sera from patients with other parasitic diseases (final diagnosis of patients), comprised of 8 cases of hepatic amebiasis, 5 of strongyloidiasis, 1 *Loa loa* filariasis, 3 neurocysticercosis, 13 toxocariasis, 5 schistosomiasis, and 8 hepatic fasciolosis;
- 55 sera from patients with non-parasitic hepatic diseases, comprised of 13 cases of hepatic carcinoma, 19 of cirrhosis, 9 auto-immune hepatitis, 6 Caroli diseases, and 8 of other liver diseases (bacterial liver abscess, simple hepatic cyst or angioma);
- 7 sera with rheumatoid factor.

Probable cases of echinococcoses are patients with positive serology and imaging. Confirmed cases will have a positive histopathological and/or a molecular diagnosis but may be from patients with negative serology (Brunetti et al., 2010). All other definitive diagnoses, i.e. for diseases other than CE or AE, were based on several criteria (gold-standard laboratory techniques, physical examination and/or imaging techniques). Patient serum samples were obtained from the Parasitology & Mycology Department, University Hospital of Besançon, France, and from the Parasitology & Mycology Department of Mustapha University Hospital, Algiers, Algeria.

2.1.2. Prospective cohort

Over a period of 4 months, at the University Hospital of Besançon, France, all patients with an echinococcosis analysis request, and with no pre-existing echinococcosis diagnosis were recruited, thus reaching a total of 115 patients.

2.2. Serology analysis

2.2.1. Standard laboratory diagnostics

Samples from both cohorts were analyzed with 3 first-line tests: indirect hemagglutination (Fumouze, Levallois Perret, France) with a lowered cut-off of ≥80 (Bart et al., 2007); *Echinococcus granulosus* ELISA (Bordier Affinity Products, Crissier, Switzerland) with a lowered cut-off OD ratio of 0.9; and Em2plus ELISA (Bordier Affinity Products) with a lowered cut-off OD ratio of 0.6. Apart from the modified cut-offs, all tests were performed according to the manufacturer's recommendations. If at least one first-line test was positive or equivocal, an immunoblot test (Echinococcus Western Blot IgG, LDBIO Diagnostic, Lyon, France) was used to confirm the positive result, as proposed in French National Health Ministry recommendations (available on the website from French National Health Insurance at http://www.codage.ext.cnamts.fr/f_mediam/fo/nabm/DOC043.pdf) and WHO-IWGE guidelines (Brunetti et al., 2010). The combination of the tests and the use of lowered cut-off values, for IHA (Bart et al., 2007) as well for *Echinococcus granulosus* and $Em2_{plus}$ ELISA (unpublished data) optimize the sensitivity of first-line analyses. A positive immunoblot is then required to confirm a positive sample or eliminate a false positive sample.

2.2.2. Rapid diagnostic test

The VIRapid® HYDATIDOSIS (Vircell, Granada, Spain) test was performed according to the manufacturer's recommendations, and each test was read independently by 2 different operators. The test line intensity was assessed by comparison with the intensity card supplied in each kit. Five levels of color intensity can be read, ranging from 0 to 3. The cut-off value is 0.5. If the Vircell test was equivocal (a faint band, but lower than 0.5, was present), a second test was performed.

2.3. Data analysis

For both cohorts, sensitivity and specificity were calculated, followed by positive [LR^+ = sensitivity/(1 − specificity)] and negative [LR^- = (1 − sensitivity)/specificity] likelihood ratios. Concerning the retrospective cohort, 2 cases were considered: a diagnosis of echinococcosis (cystic or alveolar), and then a specific diagnosis of CE only (i.e., a patient presenting with AE and testing positive would be considered a false positive). All analyses used R version 3.2.3 (URL https://www.R-project.org/, R Foundation for Statistical Computing, Vienna, Austria.),