Performance of the commercially available SERION ELISA classic Echinococcus IgG test for the detection of cystic echinococcosis in clinical practice

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Abstract

Dagnosis of cystic echinococcosis (CE) is at present mainly based on imaging techniques. Serology has a complementary role, partly due to the small number of standardized and commercially available assays. Therefore we examined the clinical performance of the SERION ELISA classic Echinococcus IgG test. Using 10 U/ml as a cut-off point, and serum samples from 50 CE patients and 105 healthy controls, the sensitivity and specificity were 98.0% and 96.2%, respectively. If patients with other infectious diseases were used as negative controls, the specificity decreased to 76.9%, which causes poor positive predictive values. However, if results between 10 and 15 U/ml are classified as indecisive, the specificity of positive results (≥15 U/ml) increased to 92.5% without greatly affecting the sensitivity (92.0%). Using this approach in combination with imaging studies, the SERION ELISA classic Echinococcus IgG test can be a useful aid in the diagnosis of CE.

Introduction

Cystic echinococcosis (CE) is a severe zoonotic disease caused by the cestode species Echinococcus granulosus, which affects livestock, wildlife and humans. The burden of CE is estimated to cause up to 1 million disability-adjusted life years and an annual loss of up to USD 2 billion in livestock production (Budke et al., 2006; Torgerson et al., 2015). When eggs of E. granulosus are ingested by humans or another intermediate host, oncospheres hatch and penetrate the intestinal mucosa. Migration to internal organs such as the liver follows, where subsequently a fluid-filled cyst develops (Eckert et al., 2001). In general, only large cysts are able to induce clinical symptoms in humans. As a consequence, most human CE cases are found incidentally during imaging examinations made for other reasons or during community screenings in endemic regions (Ammann and Eckert, 1996; Del Carpio et al., 2012).

Imaging modalities, including ultrasonography (US), CT and MRI, are important for the diagnosis of CE, which is standardized by the World Health Organization Informal Working Group on Echinococcosis in the classification of CE cysts (WHO Informal Working Group, 2003). Classification, staging, identification of possible complications and treatment response are often evaluated with the use of these imaging techniques (Polat et al., 2003). Determination of specific antibodies against E. granulosus has only a complementary role in the diagnosis of CE, because all serology tests for CE have an implicit low specificity due to cross-reactions with a plethora of diseases (Brunetti et al., 2010). However, serology can provide valuable information if imaging studies are inconclusive (Brunetti et al., 2010). Furthermore, serological response in human disease has been linked to CE-cyst stage, as patients with CE1 and CE4–CE5 cysts tend to be seronegative in a high percentage of cases, whereas patients with CE2 and CE3 cysts are often seropositive (Lissandrin et al., 2016).

Most available serological methods to detect CE lack standardization, which prevents their use as a robust diagnostic tool for CE (Manzano-Roman et al., 2015). Furthermore, the quality of commercially available ELISAs has been studied infrequently and results are rather variable, as the reported sensitivity (Se) and specificity (Sp) of these ELISAs varied from 69.5–93.14% and 76.0–98.42%, respectively (Kalantari et al., 2010; Marinova et al., 2011; Tamozzini et al., 2013, 2016; Tamer et al., 2015; Paganuzzi et al., 2016). Additionally, most studies on commercial ELISAs for detection of CE have focused on healthy controls and a wide range of infectious disease controls (Kalantari et al., 2010; Marinova et al., 2011; Tamer et al., 2015), hepatic...
cyst controls (Tamarozzi et al., 2016), or comparison with locally
developed ELISAs (Tamarozzi et al., 2013; Pagnozzi et al., 2016).
In these studies, cross-reactivity with helminth infections has been
described, but not studied extensively. In order to extend the
knowledge on both the clinical performance of commercially
available serological assays to detect CE, and infectious diseases
that cause cross-reactivity in these serological assays, we examined
the SERION ELISA classic Echinococcus IgG test.

Materials and methods

Serum samples

The present study included serum samples from 50 patients with
CE that presented at the Harbor Hospital in Rotterdam, The
Netherlands. The diagnosis of CE was proven either by micro-
scopic analysis of obtained cyst fluid (n = 8) or by characteristic
abnormalities in diagnostic imaging (ultra-sound echography,
CT-scan and/or MRI-scan) in combination with the demonstra-
tion of specific antibodies against Echinococcus spp. (n = 42).
The presence of specific antibodies against Echinococcus species
was demonstrated by two or more of the following tests: indirect
haemagglutination (IHA, Fumouze, France), ELISA using
E. granulosus cyst fluid antigen, IgG1 and IgG4 Western blot, immuno-
electrophoresis (IE) or complement binding reaction tests (CBR)
(supplementary table S1). Apart from the commercially available
indirect haemagglutination assay (van Doorn et al., 2007), all
other assays were in-house developed tests by the Dutch referral
centre for echinococcosis, the National Institute for Public
Health and the Environment (Bilthoven, the Netherlands).
Based on these imaging and serological results in combination
with clinical follow-up, the final diagnosis of CE was made by a
multidisciplinary team of clinicians, including infectious disease,
surgery, parasitology and radiology specialists.

Serum samples from 105 healthy German blood donors were
used as healthy controls, and sera from 173 patients were included
to test for cross-reactivity. The latter group included samples from
patients with several helminthiasis (ascariasis, hookworm infection,
larva migrans infection, schistosomiasis, strongyloidiasis,
toxocariasis and trichuriasis), bacterial infections (campylobacter-
iosis, rickettiosis, salmonellosis, shigellosis, shiga toxin-producing
Escherichia coli (STEC) infection, syphilis, typhoid fever) and
other infectious diseases (dengue fever, P. falciparum).

SERION ELISA classic Echinococcus IgG test

The CE-marked SERION ELISA classic Echinococcus IgG test
was performed manually according to the manufacturer’s instruc-
tions (Institut Virion|Serion GmbH, Würzburg). The antigen is
based on cyst fluid of Echinococcus granulosus. SERION ELISA
classic Echinococcus IgG is a qualitative and quantitative
immunoassay for detection of human antibodies directed against
Echinococcus granulosus and Echinococcus multilocularis. This
study, however, investigated only the clinical performance for
the detection of E. granulosus specific antibodies.

All serum samples were examined in duplicate. Optical density
(OD) signals were converted to IgG antibody concentrations (U/
ml) by a non-linear function. The quantification is based on the
4PL method, which optimizes accuracy and precision over the
maximal usable calibration range. Lot-specific 4 PL standard
curves for each SERION ELISA immunoassay are established and
four parameters are depicted on the control certificate. Test

level is adjusted by implementing a correction factor, and the
use of a single test-specific calibrator compensates for inter-assay
variations (1-Point Calibration). Echinococcus specific IgG con-
centration results were processed in Graphpad 7.0.

Results and discussion

Sensitivity and specificity of SERION ELISA classic
Echinococcus IgG test

Forty-nine of the 50 serum samples from CE patients yielded a
result above the strict diagnostic threshold of 10.0 U/ml. Of the
105 tested samples of the healthy controls, four samples tested
positive using this cut-off. Sensitivity and specificity calculated
with these groups were 98.0% and 96.2%, respectively (table 1).
Outcomes of our other serological tests are presented in supple-
mentary table S1. Our results suggest that the SERION ELISA
classic Echinococcus IgG test performs adequately, with character-
istics at least comparable to other serological methods for CE (Se
69.5–93.14% , Sp 76.0–98.42%) (Kalantari et al., 2010; Marinova
et al., 2011; Tamarozzi et al., 2013, 2016; Tamer et al., 2015;
Pagnozzi et al., 2016).

Cross-reactivity

In clinical practice, the reference population is one with a variety
of possible infectious diseases, with its inherent risk of cross-
reactivity. For this reason an additional control group of patients
with infectious diseases other than CE was introduced. When this
group was used as a negative control, the specificity of the
assay decreased to 76.9% if the cut-off of 10.0 U/ml was used
(table 1). Cross-reactivity was seen in 23.1% of the sera of the
infectious diseases control group. When the infectious diseases
were divided into subgroups, cross-reactivity was shown in
34.1% of the patients with helminthiasis, whereas cross-reactivity
was found in 7.8% of the tested sera of patients with bacterial
infections. In particular, high cross-reactivity was present in
sera from patients with strongyloidiasis, with positive SERION
ELISA classic Echinococcus IgG test results in 11 out of 12
patients (91.7%). This high percentage of cross-reactivity in
patients with helminth infections was also reported in other stud-
ies that evaluated an ELISA for the detection of CE (Brunetti
et al., 2010; Marinova et al., 2011; Tamer et al., 2015). It is
possible that the high cross-reactivity may be partially due to actual,
but so far unnoticed, CE infections in patients allocated within
the other helminth infections group, as regions where CE is
endemic overlap with regions where other helminth infections
are endemic.

The prevalence of CE varies widely between geographical
areas, with very low prevalence in northern and central
European countries and high endemicity in areas of southern
and eastern Europe (Grosso et al., 2012). If a test with a specificity
of 76.9% in a clinical control group is used, the positive predictive
value (PPV) of this test will be poor (Torgerson and Deplazes,
2009). However, when an additional classification is made to dis-
tinguish indecisive results, the specificity, and with it the PPV, can
be improved (see below).

Classification of indecisive results

Most sera with cross-reacting antibodies were only weakly posi-
tive, with levels just above 10.0 U/ml (fig. 1). Therefore, when an
additional classification was applied, marking test results
between 10 and 15 U/ml as indecisive, this improved the specificity of the test. When healthy blood donors were used as negative controls, the sensitivity and specificity of positive test results (≥15 U/ml) were 92.0% and 97.1%, respectively. When the other infectious diseases were used as negative controls, with the same threshold of 15 U/mL, specificity increased from 76.9% (with a cut-off of 10 U/ml) to 92.5%, with a sensitivity of 92.0%. A substantial number of serum samples from the infectious diseases control group that were deemed positive with the strict cut-off of 10 U/ml were reallocated to the indecisive section (n = 26; 65.0%), whereas only a few samples from patients with CE were reallocated (n = 3; 6.1%). This improvement in specificity has a positive effect on the PPV of the test, which is especially important when the prevalence of a disease is very low. Therefore, the use of an indecisive section will improve test properties considerably when used in areas with a low prevalence of CE, such as northern and western Europe.

Although the test properties of the SERION ELISA classic Echinococcus IgG are adequate, it is always essential to perform imaging studies, as this improves the PPV substantially (Torgerson and Deplazes, 2009).

**Limitations**

One constraint of our study is that the definitive diagnosis of CE for the majority of cases was made by a multidisciplinary team of experts based on clinical findings, imaging and serology, whereas other studies used microscopic verification. However, the combination of clinical findings, imaging, serology and expert opinion is currently the standard to diagnose CE, and clinical follow-up did not reveal misdiagnosis in any of the cases included in this study.

**Clinical interpretation**

In conclusion, our results demonstrated that the SERION ELISA classic Echinococcus IgG test possesses characteristics comparable to other serological methods and can be used in clinical practice to aid in diagnosing CE. In low-prevalence settings we recommend...
the following cut-off values for interpretation: negative (<10 U/ml), indecisive (≥10 and <15 U/ml) and positive (≥15 U/ml). Clinical interpretation of this test, as well as all other serological methods for CE, should always be performed in combination with imaging studies, as false-negative serology results are often obtained for patients with fully calcified cysts (Lissandrin et al., 2016) and false-positive serology results can be caused by other (helminth) infections (Tamarozzi et al., 2013; Pagnozzi et al., 2016).

**Supplementary material.** To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X18000536

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**Conflict of interest.** None.

**Ethical standards.** Ethical clearance for the storage and use of residual serum samples for scientific purposes was granted by the Medical Ethics Review Board of the Erasmus University Medical Center Rotterdam, the Netherlands (MEC 2012-047 and MEC-2015-306).

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