# Serological diagnosis in hydatidosis: Searching standardized antigenic support. Systematic review and meta-analysis

**Ayelen Rocío Retamozo**[1]
**Facundo Ariel Agüero**[2,3]
**Alicia Graciela Fuchs**[2,4]
**Andrea Maglioco**[2,3]

[1] Universidad Abierta Interamericana. Facultad de Medicina y Ciencias de la Salud. Buenos Aires, Argentina.

[2] Universidad Abierta Interamericana. Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS). Buenos Aires, Argentina.

[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

[4] Instituto Nacional de Parasitología, ANLIS "Carlos G. Malbrán", Argentina.

✉ Estados Unidos 929, C1101AAS / andrea.maglioco@uai.edu.ar

Fecha de recepción: febrero de 2021.
Fecha de aceptación: junio de 2021.

## ABSTRACT

**Background:** Hydatidosis is a worldwide distributed parasitic zoonosis caused by *Echinococcus granulosus s.l.* In humans, the diagnosis is based on epidemiological nexus, clinical findings, ultrasound images, and second-line serology. Hydatid fluid is the most widely used source of antigens in serology, but it shows high variability. This review analyzes the diagnostic utility of serology for the diagnosis of hydatidosis, particularly ELISA and Western Blot techniques. **Material and methods:** Systematic review and meta-analysis of studies with data of diagnostic validity. The reference diagnosis was ultrasound and / or parasitological medical finding. The reproducibility in the studies selection and data extraction was guaranteed, the quality was evaluated according to the QUADAS-2 guide, global results and SROC were obtained under a random effects model in Meta-Disc1.4. **Results:** Forty-one articles published between 1981 and 2019 were included. Elisa was performed in 33 articles; Western Blot was performed in 14 articles and some of the articles included both techniques. The included articles presented high heterogeneity. The SROC parameters were AUC = 0.9511 and Q=0.8919 for ELISA and AUC=0.9693 and Q = 0.9186 for Western Blot. **Conclusion:** ELISA and Western Blot have good diagnostic accuracy.

## KEYWORD

Echinococcus granulosus, Serology, Human, Diagnostic of hydatidosis, Hydatid cysts

# Diagnóstico serológico en hidatidosis: en búsqueda de un soporte antigénico estandarizado. Revisión sistemática y meta-análisis

## RESUMEN

**Introducción:** La hidatidosis es una zoonosis parasitaria mundialmente distribuida causada por el *Echinococcus granulosus s.l.* En el hombre, el diagnóstico se basa en el nexo epidemiológico, hallazgos clínicos, imágenes ecográficas y en segunda línea por la serología. El líquido hidatídico es la fuente de antígenos más utilizada en la serología, pero presenta alta variabilidad. Esta revisión analiza la utilidad diagnóstica de la serología para el diagnóstico de hidatidosis, en particular las técnicas de ELISA y Western Blot. **Materiales y métodos:** Revisión sistemática y meta-análisis de estudios con datos de validez diagnóstica. El diagnóstico de referencia fue la ecografía y/o la anatomopatología de material parasitario. Se garantizó la reproducibilidad en la selección y extracción de datos, se evaluó la calidad según la guía QUADAS-2 y se realizaron los análisis de resultados globales y SROC bajo un modelo de efectos aleatorios en Meta-Disc1.4 **Resultados:** Se incluyeron 41 artículos publicados entre 1981 y 2019. Se obtuvieron 33 artículos que utilizaron ELISA, 14 trabajos que utilizaron Western Blot y algunos de ellos utilizaron ambas técnicas. Los trabajos presentaron alta heterogeneidad. Para ELISA se obtuvo AUC=0,9511 y Q=0,8919 y para Western Blot AUC=0,9693 y Q=0,9186 en SROC. **Conclusión:** ELISA y Western Blot tienen una buena utilidad diagnóstica.

## PALABRAS CLAVE

Echinococcus granulosus, Serología, Humanos, Diagnóstico de hidatidosis, Quiste hidatídico

## INTRODUCTION

Hydatidosis or cystic echinococcosis (CE) is a parasitic zoonosis worldwide distributed (1) caused by *Echinococcus granulosus* sensu lato. This parasite has a complex life cycle with different hosts. The adult *Echinococcus granulosus* s.l., hermaphrodite worm, develops in the intestine of the definitive host (domestic dogs and wild canids) and releases eggs into the environment in the feces. The intermediate host (cattle, sheep, goats, pigs, and camelids) is infected by ingesting the oncosphere that penetrates through the intestinal epithelium and the circulatory system distributes the parasite to different organs where develops the hydatid cyst. The definitive host is infected by eating the infected viscera of the intermediate host, closing the parasite cycle. Humans are accidental intermediate hosts and become infected by taking contaminated food or water (1,2). The hydatid cyst is constituted by two layers from the parasite: the external acellular laminated layer and the inner germinal layer where protoscoleces (Pe) sprout by asexual reproduction releasing into the hydatid fluid (HF). A third layer is the consequence of the host tissue reaction, it is fibrous, avascular, with or without the presence of lymphoplasmacytic infiltration (3).

Hydatid cysts can develop in any organ, being the liver and lungs the most frequent localization (2). Eight genotypes of *Echinococcus granulosus* (G1, G3-G8, and G10) can be differentiated based on mitochondrial DNA. The genotypes most frequently found in Argentina are G1 associated with infection in sheep, G5 associated with cattle, G6 associated with camelids, and G7 associated with pigs (4,5).

This disease affects 2-3 million people worldwide and produces costs in public health and the livestock industry. The annual cost per surgical patient is 4605 USD and 251 USD for non-surgical patients (6).

In Argentina, CE is an endemic parasitosis, that is weekly and individual notifiable disease; approximately 500 new cases are reported annually in humans (7). The diagnosis of CE is mainly based on the association of clinical symptoms with epidemiological data, the finding of hydatid cyst by imaging and the detection of the disease by serological tests. The specific diagnoses of CE infections are based on micros-

copy examination of Pe, hooklets, or fragments of the parasitic membrane. The CE was staged by Gharbi according to pathognomonic images obtained by ultrasound (8) and then the WHO informal working group (9) proposed its modification. Ultrasound has a high sensitivity for hepatic cysts and determines the therapeutic guidelines: surgery, percutaneous drainage, and the use of antiparasitic drugs (2). In some cases, hydatid cysts in the early stages are not detected by ultrasound and in other cases, the images are confusing hindering the diagnosis. In endemic areas, epidemiological surveys by ultrasound scans are performed for case detection mainly in children and serology helps to confirm the diagnosis. The limitations of ultrasound are people examined per time and CE localization in non-hepatic organs but the serology has false positives and negatives. Serological diagnosis in CE involves the recognition of parasitic antigens by antibodies. The most commonly used tests are ELISA and Western blot (10, 11), being Western blot the confirmatory technique when ELISA results and images are contradictory. The antigens most commonly used for CE diagnosis are related to the hydatid cyst, particularly the HF itself (crude antigens or semi-purified antigens), or its antigens obtained by recombinant or synthetic methods. The major antigenic components are antigen B (AgB) and antigen 5 (Ag5) (12). The AgB is involved in the modulation of host immunity (13) and its structure varies according to the genotype of the parasite (14); Ag5 has an important role in the humoral response in CE1, CE4 and CE5 stages (15). The HF is a non-standardized antigenic source because its composition varies according to the stage and the localization of the cyst and the characteristics of the host (15). New antigenic options are constantly being evaluated to standardize and to increase the sensitivity and specificity of the CE serological diagnosis. This meta-analysis aimed to evaluate the diagnostic utility of serology, obtained by ELISA and Western Blot techniques.

## MATERIALS AND METHODS

### DESIGN

Systematic review and meta-analysis.

### SEARCH STRATEGY AND STUDY SELECTION.

The search was performed in PubMed (https://pubmed.ncbi.nlm.nih.gov) and Google Scholar (https://scholar.google.es/schhp?hl=es) databases on May 26, 2020. The search was conducted using the following terms:

- ("*echinococcus granulosus*" OR hydatidosis OR "cystic echinococcosis") AND (IgG OR serology) for PubMed filtering by species: human and language: English.
- ("*echinococcus granulosus*" OR hydatidosis) AND (IgG OR serological diagnosis) for Google Scholar filtering by language: Spanish and manually selecting those articles that used human sera.

### ELIGIBILITY CRITERIA FOR INCLUSION IN THE META-ANALYSIS.

1. Serological studies performed with ELISA and Western Blot techniques.
2. Serological studies detecting total IgG using HF, Pe, AgB, Ag5, or their recombinant or synthetic derivatives.
3. Studies carried out on patients with a diagnosis of CE confirmed by ultrasound imaging and/or pathological examination of parasitic material; without a restriction of years of publication.

### ELIGIBILITY CRITERIA FOR EXCLUSION FROM THE META-ANALYSIS.

1. Studies that do not confirm the presence of the disease by ultrasound imaging and/or pathological examination of parasitic material.
2. Studies specifying that patients have been treated medically or surgically before sample extraction.
3. Studies evaluating ELISA and/or Western Blot in samples chosen for their positivity to other serological techniques.
4. Studies that do not provide any data to obtain true positives, true negatives, false negatives, and false positives values.
5. Studies reporting fewer than 10 patients.

6. Studies that the full text is not accessible in English or Spanish.

### REPRODUCIBILITY IN THE STUDY SELECTION AND THE DATA COLLECTION

Studies were independently selected and assessed by two reviewers to ensure the reproducibility of studies selection and data collection.

### EVALUATION OF THE QUALITY OF THE STUDIES

The Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS-2) was used to evaluate the quality of the included studies (16).

### DATA ANALYSIS

A 2x2 table was constructed with the data of true positives, true negatives, false positives, and false negatives reported in each paper or calculated with the data provided by the authors.
All analyses were performed under the random-effects model in the Meta-DiSc 1.4 software (17) adding 0.5 to the cells of studies with zero. Sensitivity and specificity values and their 95% confidence intervals (95% CI) were calculated for each study. The pooled sensitivity and specificity values were obtained for Western blot and ELISA, analyzed together or individually, and for the most frequent localization for hydatid cyst (liver and lung). The heterogeneity of sensitivity and specificity results was evaluated by forest plot and the chi-square statistic ($p<0.05$) and the inconsistency by $I^2$.
The diagnostic accuracy of the different serological methods was evaluated by analysis of the area under the curve (AUC) SROC (summary receiver operating characteristics) and the Q index; by the DerSimonian-Laird method.

## RESULTS

### SELECTION OF STUDIES

A total of 1613 studies were identified and no duplicate studies were found among the databases used (PubMed and Google Scholar). Inclusion and exclusion criteria were applied for manual filtering according to the title and abstract, 1436 papers were excluded. A total of 177 full articles were reviewed and 41 articles were included in the review and meta-analysis (Figure 1).

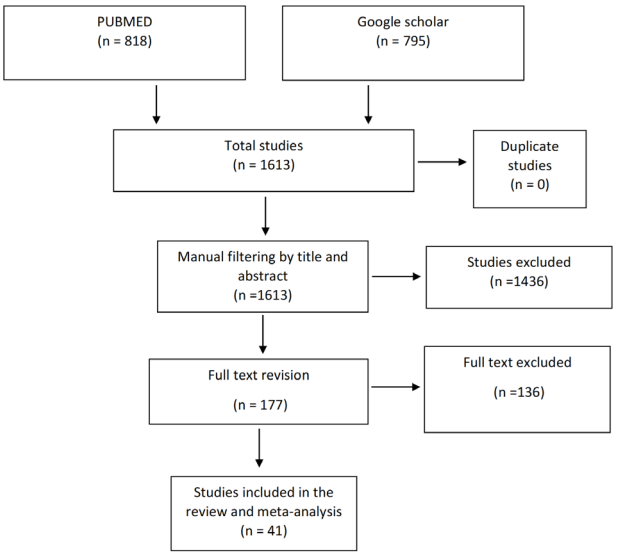



FIGURE 1. FLOW CHART SUMMARIZING THE LITERATURE SEARCH AND STUDY SELECTION.

## DESCRIPTION OF SELECTED STUDIES

The articles included in the review and meta-analysis were published between 1981 and 2019 and they evaluated the serological diagnosis of CE by the detection of IgG antibodies using different antigenic sources. Articles had differences in the quality of the samples used as controls: samples from healthy donors, samples from patients with non-parasitic diseases, or samples from patients with other parasitoses.

Regarding the source of the antigens used for the serological analysis, 27 studies used HF or Pe, 6 studies used purified HF antigens, 10 studies used commercial products, and 6 studies used recombinant or synthetic antigens. Thirty-three studies used ELISA, 14 studies used Western Blot, and some of these studies used both techniques. The age of the patients was reported in 13 studies, the cyst stage in 4 studies, and the cyst localization in 25 studies (Table 1).

| REFERENCE | ANTIGEN | SEROLOGICAL METHOD | AGE | CYST STAGE | CYST LOCALIZATION |
|---|---|---|---|---|---|
| Miranda E. et al. (2010) (19) | LH (goat) 21-31 kDa | Western Blot | Data | No data | Data |
| | LH (ovine) 21-31 kDa | Western Blot | | | |
| Gómez TPJC (2004) (20) | LH (ovine) 21-31 kDa | Western Blot | No data | | |
| | LH (bovine) 38 kDa | Western Blot | | | |
| | LH (human) 38 kDa | Western Blot | | | |
| Hernández A. et al. (2018) (21) | LH (ovine) | ELISA | No data | Data | |
| | AgB2t | ELISA | | | |
| | Ag2B2t | ELISA | | | |
| Tenguria RK. et al. (2014) (22) | LH (ovine) | ELISA | Data | No data | Data |
| Han X. et al. (2019) (23) | rEgAgB1 | ELISA | Data | | |
| | rEgAgB2 | ELISA | | | |
| | rEgAgB3 | ELISA | | | |
| | rEgAgB4 | ELISA | | | |
| | rEgAgB5 | ELISA | | | |
| Fathi S. et al. (2016) (24) | AgB native (commercial kit, Pishtaz, Iran) | ELISA | No data | | |
| de la Rue ML. et al. (2010) (25) | LH (ovine) 8/16/24 kDa | Western Blot | No data | | |
| Sadjjadi SM. et al. (2009) (26) | AgB native | ELISA | Data | No data | |
| Tamer SG. et al. (2015) (27) | Ags natives de E. granulosus (commercial kit, DRG, Germany) | ELISA | Data | No data | |
| Vola A. et al. (2019) (28) | Ags natives (QH) de E. granulosus (commercial kit, RIDASCREEN, Germany) | ELISA | No data | Data | |
| | Ags natives de E. multilocularis (commercial kit, LDBIO, France) | Western Blot | | | |

| REFERENCE | ANTIGEN | SEROLOGICAL METHOD | AGE | CYST STAGE | CYST LOCALIZATION |
|---|---|---|---|---|---|
| Park SJ. et al. (2015) (29) | LH (ovine) | ELISA | Data | No data | |
| Hernández A. et al. (2008) (30) | LH (ovine) | ELISA | No data | | Data |
| | AgB2t | ELISA | | | |
| Chirag S. et al. (2015) (31) | LH (ovine) | ELISA | Data | No data | Data |
| Savardashtaki A. et al. (2017) (32) | rEgAgB8/1 | ELISA | No data | | Data |
| | AgB native | ELISA | | | |
| | Ags natives de E. multilocularis (commercial kit, Euroimmun, Germany) | ELISA | | | |
| Jin Y. et al. (2013) (33) | LH (ovine) | ELISA | Data | No data | |
| Iraqi W. (2016) (34) | LH (ovine) | Western Blot | No data | | |
| | LH (ovine) | ELISA | | | |
| Reiterová K. et al. (2014) (35) | LH (ovine) | Western Blot | No data | | Data |
| | AgB native | Western Blot | | | |
| | LH (ovine) | ELISA | | | |
| | AgB native | ELISA | | | |
| Mamuti W. et al. (2002) (36) | LH (ovine) 8 kDa | Western Blot | No data | | |
| | LH (human) 8 kDa | | | | |
| | LH (mice) 8 kDa | | | | |
| | LH (ovine) | ELISA | | | |
| | LH (mice) | | | | |
| Bauomi IR. et al. (2014) (37) | 27,5 kDa Protoescólices | ELISA | No data | | Data |
| El-Shazly AM. et al. (2010) (38) | Ags natives (LH) de E. granulosus (commercial kit, Bordier Affinity Products SA; Switzerland) | ELISA | No data | | |
| Santivañez SJ. et al. (2012) (39) | P176 sinthetic (AgB8/1 N-terminal) | ELISA | Data | | |
| Eris FN. et al. (2009) (40) | LH (ovine) | ELISA | No data | | Data |
| | Ags natives de E. granulosus (commercial kit, Vircell SL, Spain) | ELISA | | | |

| REFERENCE | ANTIGEN | SEROLOGICAL METHOD | AGE | CYST STAGE | CYST LOCALIZATION |
|---|---|---|---|---|---|
| Barbieri M. et al. (1998) (41) | LH (bovine) | ELISA | No data | No data | Data |
| | AgB native | ELISA | | | |
| | Ag5 native | ELISA | | | |
| | Ag5 native (PBS) | ELISA | | | |
| | AgB native (PBS) | ELISA | | | |
| | 65-BSA (PBS) | ELISA | | | |
| | GU4 (TFE) | ELISA | | | |
| | 89-122 (TFE) | ELISA | | | |
| Shambesh MK. et al. (1995) (42) | LH (camel) 100/130 kDa | Western Blot | | No data | |
| | LH (camel) 8/16/24 kDa | Western Blot | | | |
| | LH (camel) 38 kDa | Western Blot | | | |
| Sadjjadi SM. et al. (2007) (43) | AgB native | ELISA | | No data | |
| Shapiro SZ. et al. (1992) (44) | LH comercial (BioMérieux, France) 12,5 kDa | Western Blot | | No data | |
| | 16,5-18 kDa | | | | |
| | 40 kDa | | | | |
| Wattal C. et al. (1986) (45) | LH (human) | ELISA | | No data | Data |
| | Protoescólices (SSA) | ELISA | | | |
| Barbieri M. et al. (1993) (46) | LH (bovine) | ELISA | | No data | Data |
| Grimm F. et al. (1998) (47) | LH (bovine) | ELISA | | No data | |
| Gadea I. et al. (1999) (48) | LH (human) | Western Blot | | No data | Data |
| El-Ghareeb AS. et al. (2016) (49) | Ags de Echinococcus (Commercial kit, Abcam, EE.UU). | ELISA | Data | No data | Data |
| Moghadam ZK. et al. (2013) (50) | Protoescólices 60 kDa | Western Blot | | No data | Data |
| Shweiki HM. et al. (1992) (51) | Ag native para arco-5 en IEF (Comercial, Diagnostics Pasteur-France) | ELISA | Data | No data | Data |
| Ayadi A. et al. (1995) (52) | LH comercial (BioMérieux, France) 8/21/30/35/92 kDa | Western Blot | | No data | Data |
| Poretti D. et al. (1999) (53) | LH | ELISA | No data | No data | Data |
| | LH 8 kDa | Western Blot | | | |
| | LH 8/29/34 kDa | Western Blot | | | |

| REFERENCE | ANTIGEN | SEROLOGICAL METHOD | AGE | CYST STAGE | CYST LOCALIZATION |
|---|---|---|---|---|---|
| Barbieri M. et al. (1994) (54) | HBLF (LH fraction) | ELISA | No data | | Data |
| Tawfeek GM. et al. (2011) (55) | AgB native | ELISA | No data | | |
| | LH (ovine) | ELISA | | | |
| | AgB 12kDa | ELISA | | | |
| Manterola CG. et al. (2003) (56) | LH (ovine) | ELISA | Data | No data | Data |
| | LH (ovine) | ELISA | | | |
| Guisantes JA. et. al. (1981) (18) | LH (ovine) | ELISA | No data | | Data |
| Flores LAA. et al. (2006) (57) | LH (ovine) | ELISA | No data | | |
| Manterola C. et al. (2005) (58) | LH (ovine) | ELISA | Data | No data | Data |
| | LH (ovine) | Western Blot | | | |

TABLE 1: DESCRIPTION OF THE STUDIES INCLUDED IN THE REVIEW AND META-ANALYSIS. ABBREVIATIONS: HF: HYDATID FLUID; HC: HYDATID CYST; AG: ANTIGEN; AGS: ANTIGENS; PBS: PHOSPHATE BUFFER SOLUTION; BSA: BOVINE SERUM ALBUMIN; TFE: TRIFLUOROETHANOL; IEP: IMMUNOELECTROPHORESIS.

## QUALITY OF THE SELECTED WORKS

The studies presented a satisfactory level of quality. Only one study was rated under the uncertain risk category due to the use of old methods for the technical determination of the cut-off value (18) (Figure 2).

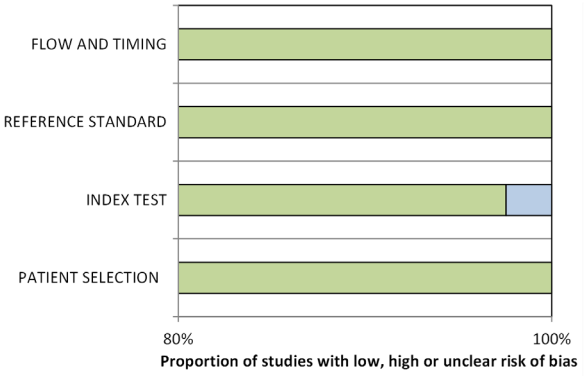
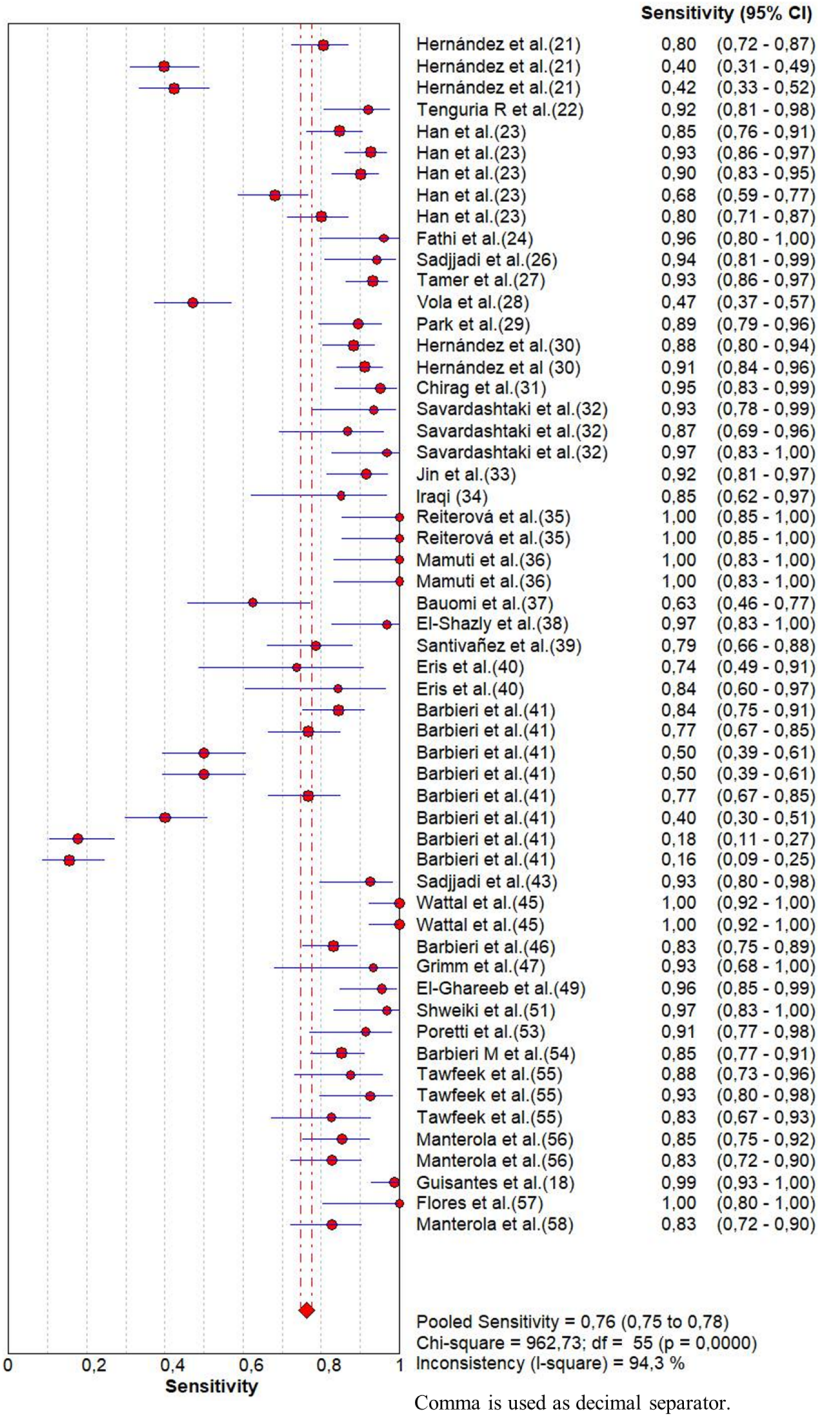



FIGURE 2. RISK OF BIAS ASSESSED BY QUADAS-2. UNCLEAR RISK IS SHOWN IN BLUE, LOW RISK IS SHOWN IN GREEN AND HIGH RISK OF BIAS IS SHOWN IN RED.

## META-ANALYSIS

All the included studies were analyzed together. The pooled sensitivity was 78% (95% CI 77-80) and I2 was 93.4 %. The pooled specificity was 90% (95% CI 90-91) and I2 was 95.9 %.




Comma is used as decimal separator.

FIGURE 3. FOREST PLOT OF SENSITIVITY FOR STUDIES USING ELISA.

## STUDIES ANALYZED BY SEROLOGICAL TECHNIQUE

A. ELISA. The sensitivity showed greater variability than the specificity. The pooled sensitivity was 76% (95% CI 75-78) and $I^2$ was 94.3%. Only 8 sensitivity values were below this result and 25 sensitivity va-

lues were above it. The study with the lowest sensitivity value was that by Barbieri M. et al. (41) using the synthetic antigens GU4 and 89-122, while the studies by Wattal C. et al. (45) and Guisantes JA. et al. (18) reported the highest sensitivity values using HF or Pe antigens (Figure 3). The pooled specificity was 89% (95% CI 89-90) and $I^2$ was 96.3%. Twelve specificity values were found below the pooled specificity value and 14 specificity values were above it. The lowest specificity values were reported by Hernández A. et al. (21) using AgB2t and Ag2B2t or HF from cyst from ovine and Mamuti W. et al. (36) using HF from ovine or from experimentally infected mice. The highest specificity values were found by Wattal C. et al. (45), Barbieri M. et al. (41) using synthetic peptide 89-122, Vola A. et al. (28) using a commercial ELISA with native antigens from *Echinococcus granulosus* cyst (RIDASCREEN, Germany) and Barbieri M. et al. (54) using an HF fraction (Figure 4). The area under the curve (AUC) of SROC for ELISA was 0.9511 and the Q index was 0.8919 (Figure 5 A)

B. Western Blot. The sensitivity values showed less variability than specificity values. The pooled sensitivity was 85% (95% CI 83-87) and $I^2$ was 88.3%. Three sensitivity values were lower than the pooled sensitivity value and 2 sensitivity values were higher. The lowest sensitivity value was reported by Moghadam ZK. et al. (50) using the 60 kDa band from Pe homogenate and the highest sensitivity values were reported by Shapiro SZ. et al. (44) and Ayadi A. et al. (52) (Figure 6) both using commercial HF (BioMérieux, France). The pooled specificity was 93% (95% CI 92-94) and $I^2$ was 94.3%. Seven specificity values were found below the pooled specificity value and four values were above it. The study with the lowest specificity value was that by Shapiro SZ. et al. (44) using the 40 kDa band from commercial HF (BioMérieux, France) as antigenic support. The highest specificity was found in the study by Vola A. et al. (28) using a commercial Western Blot (LDBIO, France) with native antigens from *Echinococcus multilocularis* but with a differential pattern of bands for *Echinococcus granulosus* detection (Figure 7). The area under the curve (AUC) of SROC for Western Blot was 0.9693 and the Q index was 0.9186 (Figure 5 B).

## STUDIES ANALYZED BY CYST LOCALIZATION

Liver. The pooled sensitivity was 75% (95% CI 72-79) and $I^2$ was 92.1%. The study by Vola A. et al (28) is the only one that presented a sensitivity value below the pooled sensitivity value in the two serological methods used: ELISA and Western Blot. The study by Guisantes J. et al. (18) and Wattal C et al. (45) presented values above the pooled sensitivity value (Figure 8 A). The pooled specificity was 95% (95% CI 94-97) and the values found by Vola A. et al. (28), Wattal C. et al. (45), and Barbieri M et al. (46) were higher than the pooled specificity value (Figure 8 B).

Lung. The pooled sensitivity was 91% (95% CI 84-96) and $I^2$was 72.8%. Results from all the studies were found within the confidence interval of pooled sensitivity value (Figure 9 A), however, the study by Guisantes J. et al (18) presented a specificity value lower than the pooled specificity value of 97% (95% CI 95-98) (Figure 9 B).

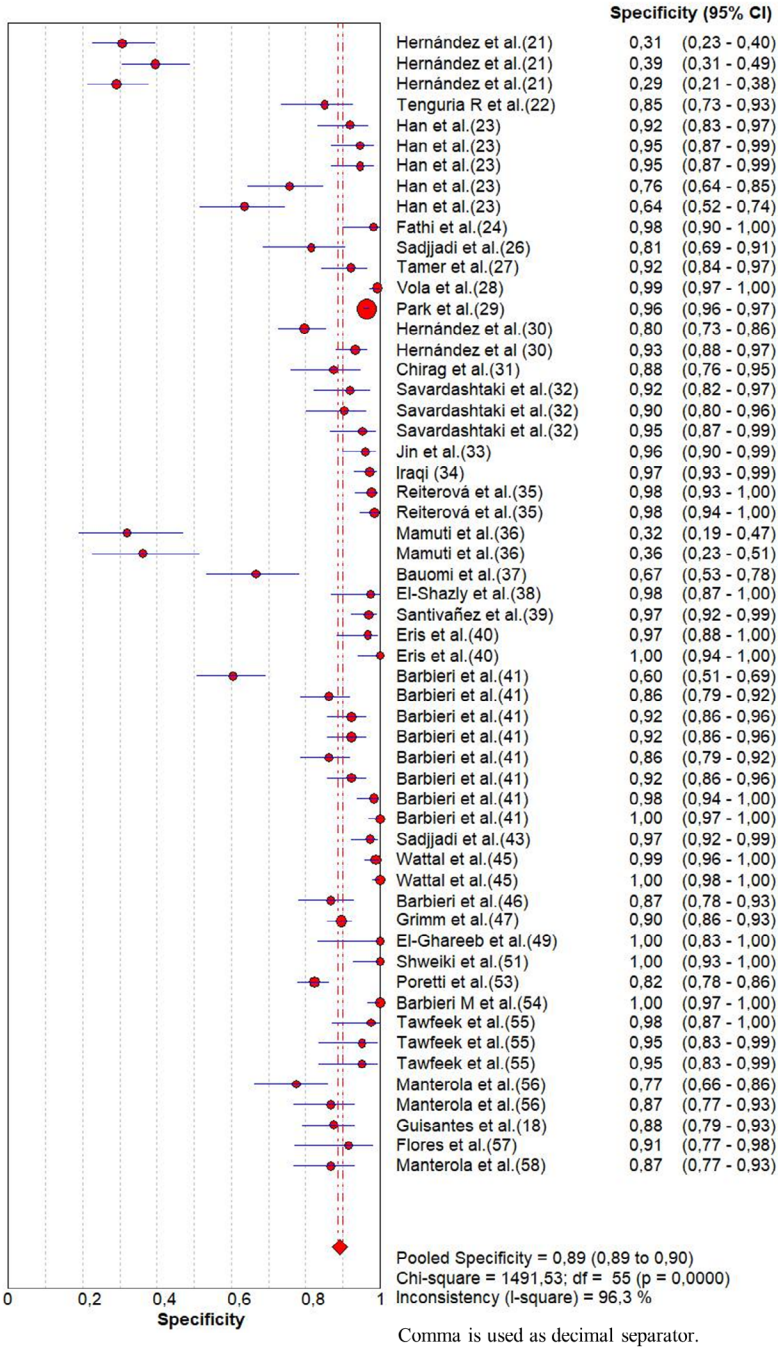



FIGURE 4. FOREST PLOT OF SPECIFICITY FOR STUDIES USING ELISA.

Summary receiver operating characteristics (SROC) for studies using A) ELISA and B) Western Blot.

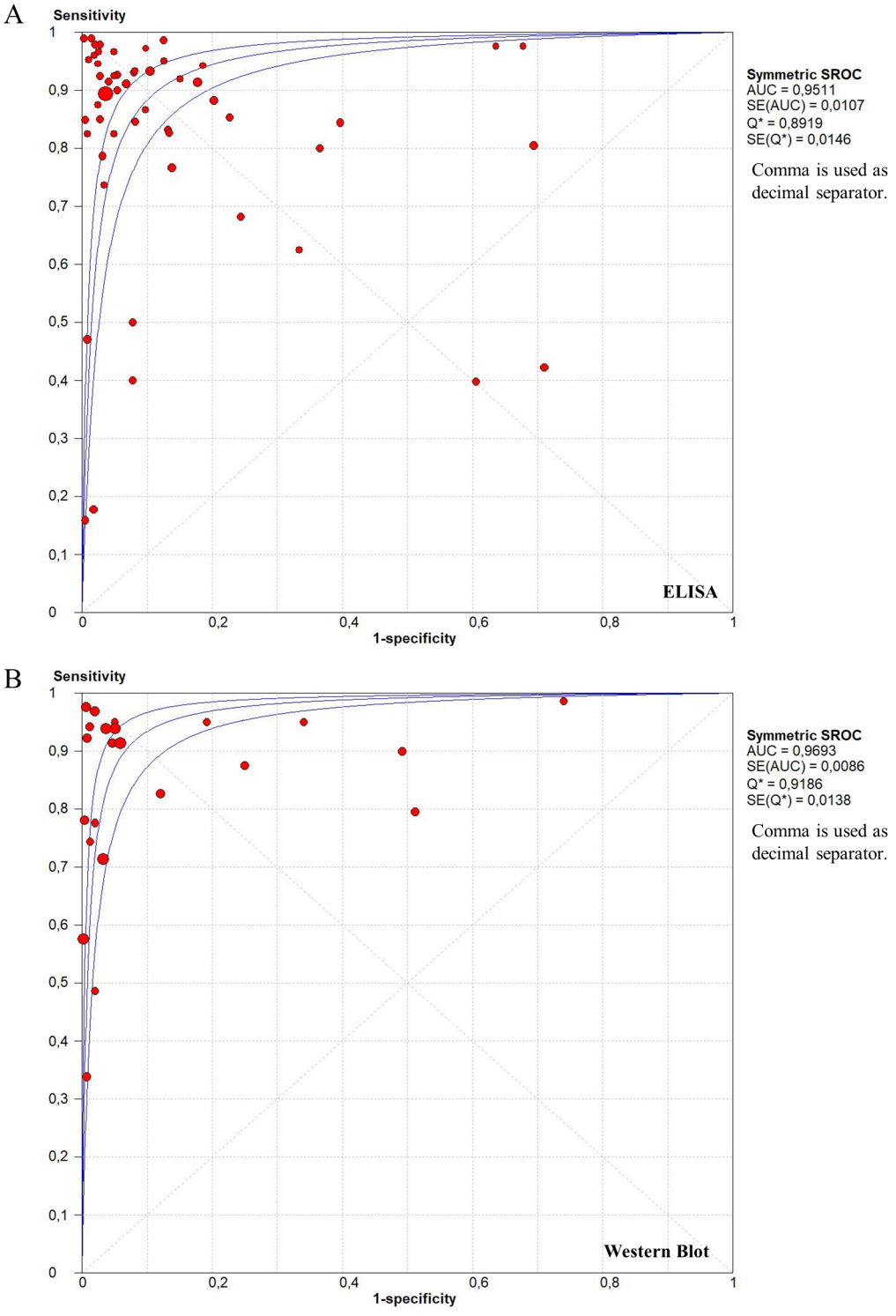



FIGURE 5. SUMMARY RECEIVER OPERATING CHARACTERISTICS (SROC) FOR STUDIES USING A) ELISA AND B) WESTERN BLOT.

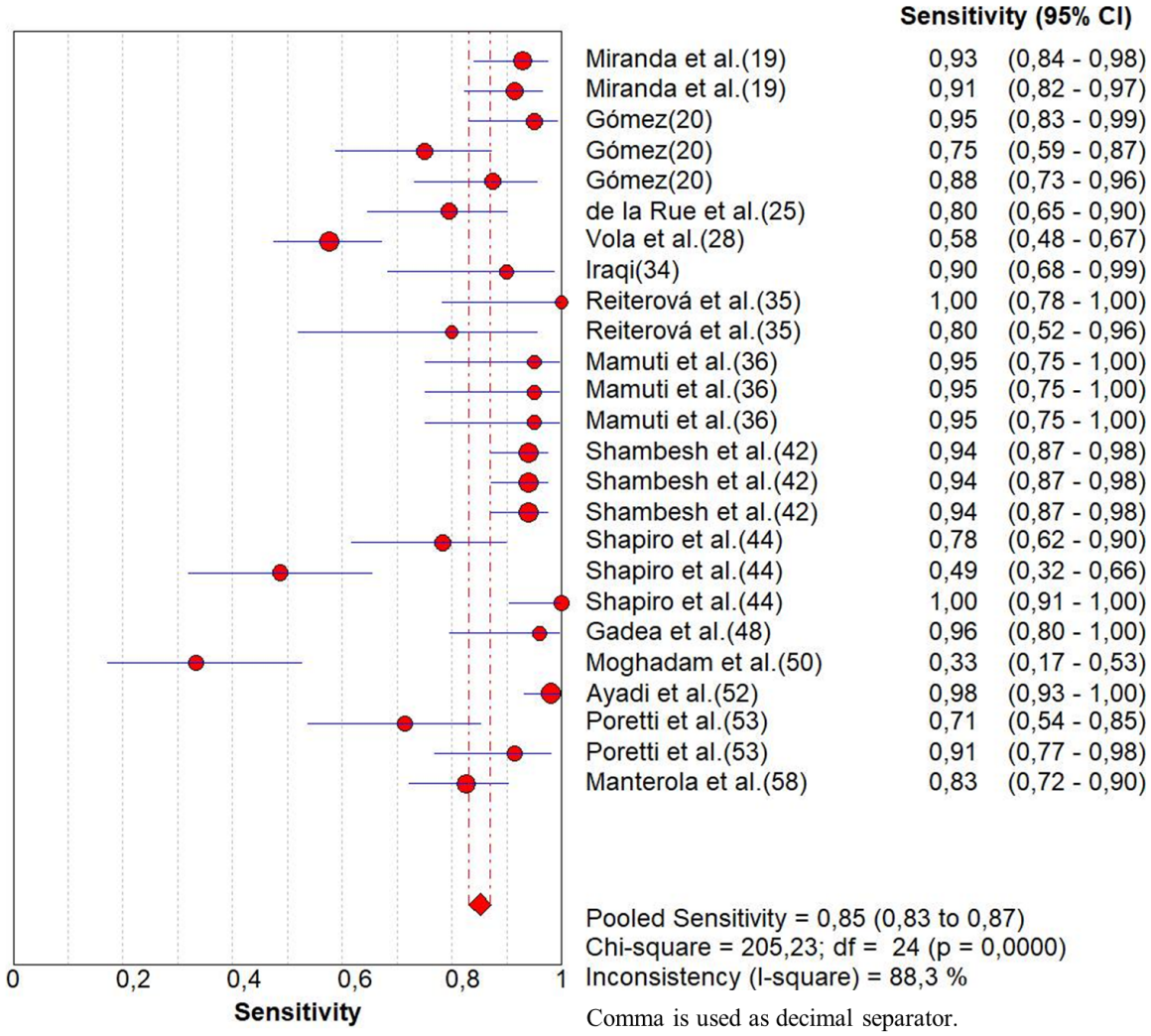



FIGURE 6. FOREST PLOT OF SENSITIVITY FOR STUDIES USING WESTERN BLOT.

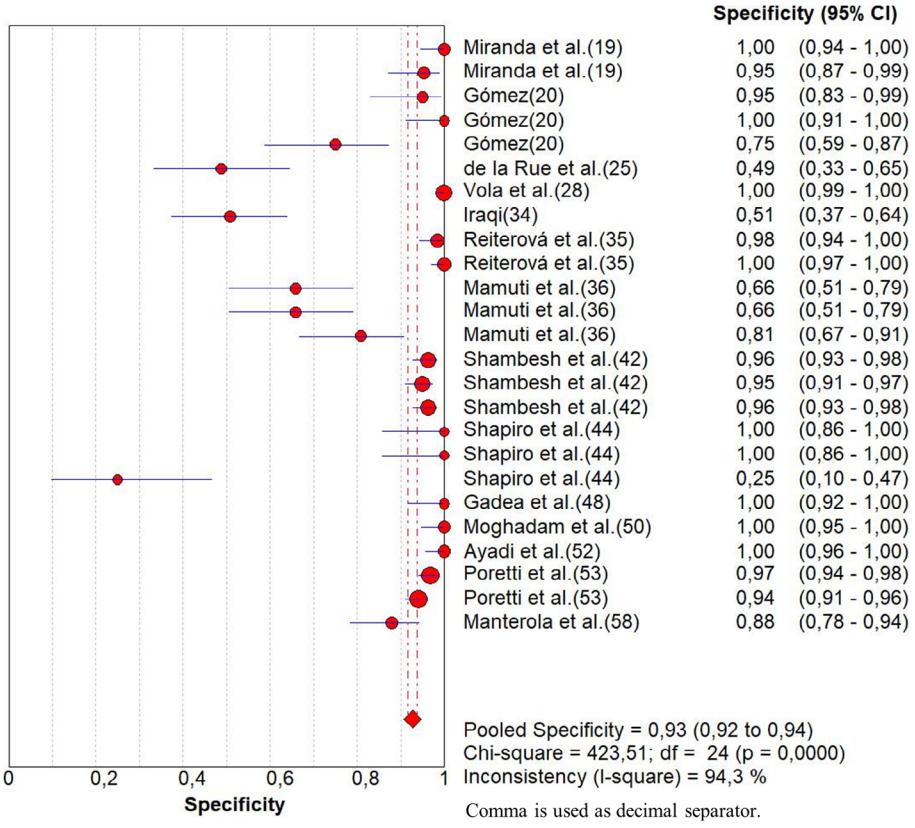



FIGURE 7. FOREST PLOT OF SPECIFICITY FOR STUDIES USING WESTERN BLOT.

## DISCUSSION

To reduce the variability observed in the serological diagnosis of CE, there are new alternatives to the standard diagnosis such as the use of different antigens as HP20 (59), the use of a cell line obtained from *Echinococcus granulosus* as a standardized source of antigens (60) or the use of biological assays that measure cytokines (61).

This meta-analysis analyzed the most commonly used serological methods for CE diagnosis, ELISA and Western Blot. It included studies testing HF from naturally or experimentally infected animals, ex-vivo commercial HF, antigens from Pe, antigens purified from parasitic material, recombinant and synthetic antigens related to Ag5 and AgB, and commercial detection kits based on identified native antigens or based on a pool of antigens from infected animals.

Most of the analyzed studies used HF or purified antigens. In the case of the studies that used HF, the reactivity to more than one antigen increased the sensitivity. In particular, the studies by Ayadi A. et al. (52) and Poretti D. et al. (53) demonstrated a positive relationship between the cases identified and the number of bands included in the analysis. Ex-vivo parasitic material used as antigenic support for serological tests is difficult to obtain, it is scarce and variable. Mamuti W. et al (36) obtained HF from experimentally infected mice and demonstrated that the sensitivity value was similar to that obtained with HF from parasitic cysts from humans or ovines.

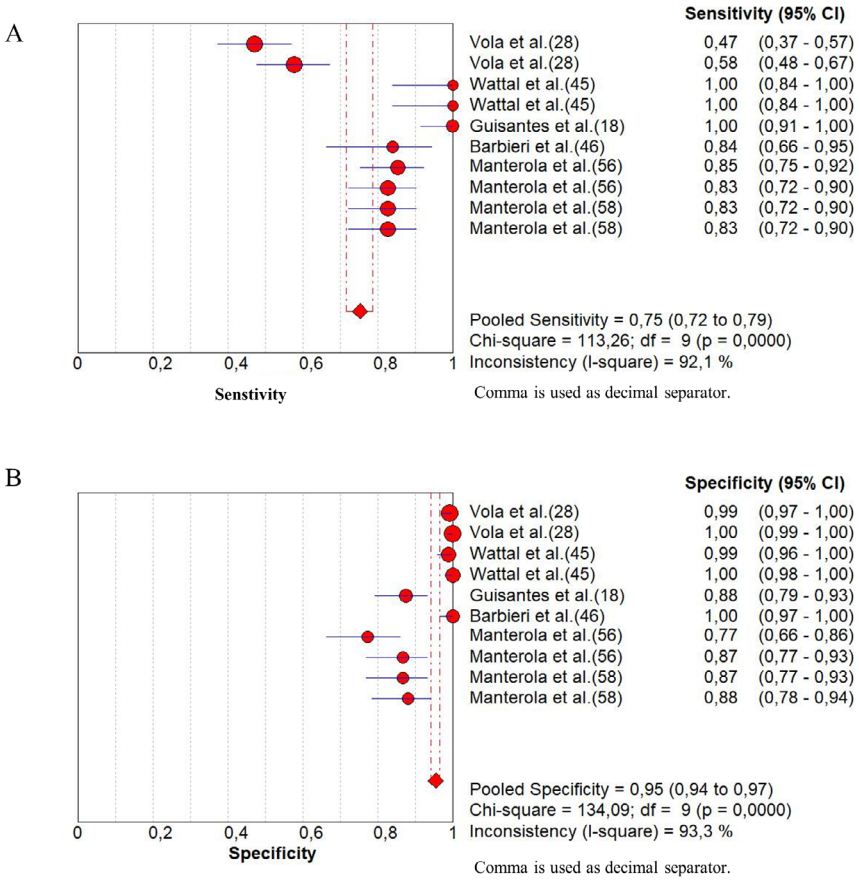



FIGURE 8. FOREST PLOT OF A) SENSITIVITY AND B) SPECIFICITY OF THE STUDIES THAT ANALYZED CYST LOCALIZED IN THE LIVER.

The standardization of the antigen source used for diagnosis, as a commercial product, showed high sensitivity and specificity, even when the source of antigen was ex-vivo parasitic material.

Cyst localization may be relevant in the host immune response due to the differences reported in the permeability (62) or the protein composition of the cyst (63) associated with cysts localization or stage. In this work, we analyzed the sensitivity and specificity of the serological diagnosis associated with the different localization of the parasite. A higher pooled sensitivity was found in the lung and no significant differences were found in the pooled specificity values. The present study did not include that CE cases without ultrasound confirmation due to the size or localization of the cyst. The analysis by anatomical localization of the cyst included only the most frequent localizations, due to the absence of enough studies for other localizations. It is important to highlight the scarce clinical information or staging of the disease in patients in each of the included studies, the variability in the controls used and the diversity of the antigenic source.

ELISA and Western Blot methods showed good diagnostic performance and accuracy in the detection of CE in patients with confirmed cyst presence. Several factors are involved in the correct serological detection of the disease, some of them intrinsic to the host and its immune response, related to the parasitic infection and the cyst localization, and others inherent to the serological diagnostic method used (11). The high heterogeneity among the studies had an impact on the estimation of the pooled sensitivity and specificity parameters. The analysis of individual studies, using different antigens, revealed that some studies presented high values of sensitivity or speci-

ficity. This indicates that serology can be a good tool for the diagnosis of the disease if the appropriate and standardized antigenic supports are used. A better understanding of the host immune response and the use of more than one antigen for disease diagnosis could improve the current serological diagnosis.

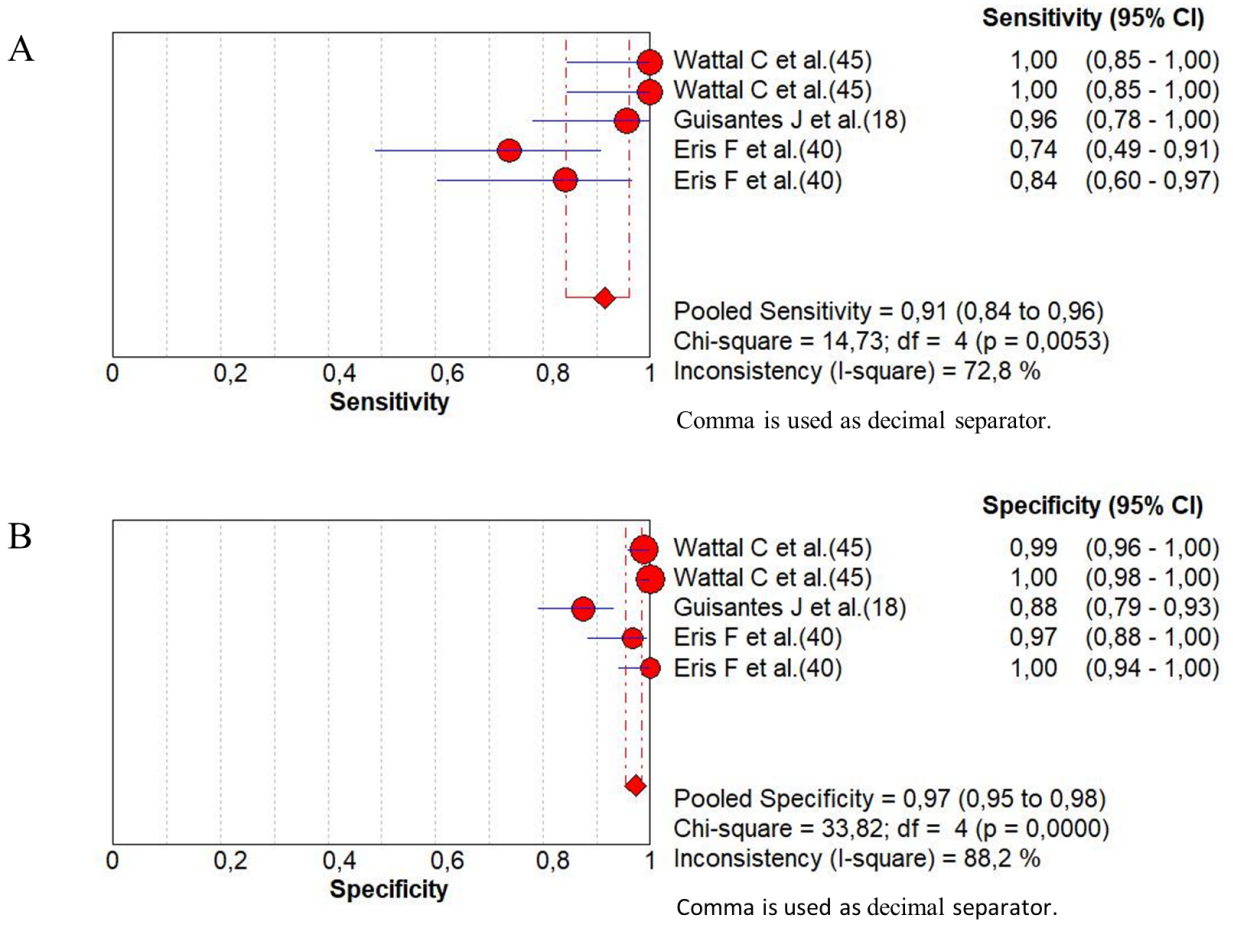



FIGURE 9. FOREST PLOT OF A) SENSITIVITY AND B) SPECIFICITY OF THE STUDIES THAT ANALYZED CYST LOCALIZED IN THE LUNG.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## REFERENCES

1. Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W, et al. Echinococcosis: Advances in the 21st Century. Clin Microbiol Rev. 2019;32(2):e00075-18. doi: 10.1128/CMR.00075-18. PMID: 30760475; PMCID: PMC6431127.
2. Armiñanzas C, Gutiérrez-Cuadra M, Fariñas MC. Hidatidosis: aspectos epidemiológicos, clínicos, diagnósticos y terapéuticos. Rev Esp Quimioter. 2015;28(3):116-24. PMID: 26032995.
3. Wu XW, Chen XL, Zhang SJ, Zhang X, Sun H, Peng XY. Pericyst may be a new pharmacological and therapeutic target for hydatid disease. Chin Med J (Engl). 2011;124(18):2857-62. PMID: 22040492.
4. Kinkar L, Laurimäe T, Sharbatkhori M, Mirhendi H, Kia EB, Ponce-Gordo F, et al. New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm Echinococcus granulosus sensu stricto. Infect Genet Evol. 2017;52:52-58. doi: 10.1016/j.meegid.2017.04.023. Epub 2017. PMID: 28456662.
5. Cucher MA, Macchiaroli N, Baldi G, Camicia F, Prada L,

Maldonado L, et al. Cystic echinococcosis in South America: systematic review of species and genotypes of Echinococcus granulosus sensu lato in humans and natural domestic hosts. Trop Med Int Health. 2016;21(2):166-75. doi: 10.1111/tmi.12647. Epub 2015. PMID: 26610060.

6. Bingham GM, Larrieu E, Uchiumi L, Mercapide C, Mujica G, Del Carpio M, et al. The Economic Impact of Cystic Echinococcosis in Rio Negro Province, Argentina. Am J Trop Med Hyg. 2016;94(3):615-25. doi: 10.4269/ajtmh.15-0304. Epub 2016. PMID: 26787144; PMCID: PMC4775899.
7. Organización Panamericana de la Salud. Equinococosis: Informe epidemiológico en la región de América del Sur - 2016-2017, n.3, 2019 [Internet]. 2019. [Citado 10 de junio de 2021]. Disponible en: https://iris.paho.org/handle/10665.2/50630
8. Gharbi HA, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. Radiology. 1981 May;139(2):459-63. doi: 10.1148/radiology.139.2.7220891. PMID: 7220891.
9. Brunetti E, Kern P, Vuitton DA; Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 2010;114(1):1-16. doi: 10.1016/j.actatropica.2009.11.001. Epub 2009. PMID: 19931502.
10. Centro Panamericano de Fiebre Aftosa. Organización Panamericana de la Salud. Organización Mundial de la Salud. Prevención y Control de la Hidatidosis en el Nivel Local: iniciativa sudamericana para el control y vigilancia de la equinococosis quística / hidatidosis. Organización Panamericana de la Salud - OPS/OMS. Río de Janeiro: PANAFTOSA - OPS/OMS, 2017.
11. Siles-Lucas M, Casulli A, Conraths FJ, Müller N. Laboratory Diagnosis of Echinococcus spp. in Human Patients and Infected Animals. Adv Parasitol. 2017;96:159-257. doi: 10.1016/bs.apar.2016.09.003. Epub 2017. PMID: 28212789.
12. Aziz A, Zhang W, Li J, Loukas A, McManus DP, Mulvenna J. Proteomic characterisation of Echinococcus granulosus hydatid cyst fluid from sheep, cattle and humans. J Proteomics. 2011;74(9):1560-72. doi: 10.1016/j.jprot.2011.02.021. Epub 2011. PMID: 21362505..
13. Riganò R, Profumo E, Bruschi F, Carulli G, Azzarà A, Ioppolo S, et al. Modulation of human immune response by Echinococcus granulosus antigen B and its possible role in evading host defenses. Infect Immun. 2001;69(1):288-96. doi: 10.1128/IAI.69.1.288-296.2001. PMID: 11119517; PMCID: PMC97883.
14. Kamenetzky L, Muzulin PM, Gutierrez AM, Angel SO, Zaha A, Guarnera EA, et al. High polymorphism in genes encoding antigen B from human infecting strains of Echinococcus granulosus. Parasitology. 2005;131(Pt 6):805-15. doi: 10.1017/S0031182005008474. PMID: 16336734.
15. Ahn CS, Han X, Bae YA, Ma X, Kim JT, Cai H, et al. Alteration of immunoproteome profile of Echinococcus granulosus hydatid fluid with progression of cystic echinococcosis. Parasit Vectors. 2015;8:10. doi: 10.1186/s13071-014-0610-7. PMID: 25566682; PMCID: PMC4311513.
16. Ciapponi A. QUADAS-2: instrumento para la evaluación de la calidad de estudios de precisión diagnóstica. Evid actual pract ambul [Internet]. 2015 [citado 10 de junio de 2021];18(1). Disponible en: http://www.evidencia.org/index.php/Evidencia/article/view/6341
17. Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Med Res Methodol. 2006;6:31. doi: 10.1186/1471-2288-6-31. PMID: 16836745; PMCID: PMC1552081.
18. Guisantes JA, Rubio MF, Díaz R. Aplicación de un método inmunoenzimático (ELISA) al diagnóstico de la hidatidosis humana. Bol Oficina Sanit Panam. 1981;90(2):160-8. PMID: 6452148.
19. Miranda E, Velarde F, Somocurcio J, Ayala E. Evaluación de dos pruebas de inmunoblot con antígeno hidatídico de goat y ovino para el diagnóstico de equinococosis humana. Rev. perú. med. exp. salud pública. 2010: 209-214.
20. Gómez TPJC. Valor diagnóstico de inmunoblot con líquido hidatídico humano frente a antígeno ovino y bovino. Rev Mex Patol Clin Med Lab. 2004;51(2):75-89.
21. Hernández-González A, Sánchez-Ovejero C, Manzano-Román R, González Sánchez M, Delgado JM, Pardo-García T, et al. Evaluation of the recombinant antigens B2t and 2B2t, compared with hydatid fluid, in IgG-ELISA and immunostrips for the diagnosis and follow up of CE patients. PLoS Negl Trop Dis. 2018;12(9):e0006741. doi: 10.1371/journal.pntd.0006741. PMID: 30188936; PMCID: PMC6143278.
22. Tenguria RK, Naik MI. Evaluation of human cystic echinococcosis before and after surgery and chemotherapy by demonstration of antibodies in serum. Ann Parasitol. 2014;60(4):297-303. PMID: 25706429.
23. Han X, Kim JG, Wang H, Cai H, Ma X, Duong DH, et al. Survey of echinococcoses in southeastern Qinghai Province, China, and serodiagnostic insights of recombinant Echinococcus granulosus antigen B isoforms. Parasit Vectors. 2019;12(1):323. doi: 10.1186/s13071-019-3569-6. PMID: 31242932; PMCID: PMC6593596.
24. Fathi S, Jalousian F, Hosseini SH, Parsa H, Kordafshari S. A Study of Cross-Reactivity Between Recombinant EPC1 Antigen of Echinococcus granulosus in Serum from Patients with Confirmed Cystic Echinococcosis Infection and Other Parasitic Infections. Am J Trop Med Hyg. 2016;94(6):1313-7.

doi: 10.4269/ajtmh.15-0680. Epub 2016. PMID: 27091868; PMCID: PMC4889750.

25. de la Rue ML, Yamano K, Almeida CE, Iesbich MP, Fernandes CD, Goto A, et al. Serological reactivity of patients with Echinococcus infections (E. granulosus, E. vogeli, and E. multilocularis) against three antigen B subunits. Parasitol Res. 2010;106(3):741-5. doi: 10.1007/s00436-009-1707-3. PMID: 20066435.
26. Sadjjadi SM, Sedaghat F, Hosseini SV, Sarkari B. Serum antigen and antibody detection in echinococcosis: application in serodiagnosis of human hydatidosis. Korean J Parasitol. 2009;47(2):153-7. doi: 10.3347/kjp.2009.47.2.153. Epub 2009. PMID: 19488422; PMCID: PMC2688797.
27. Tamer GS, Dündar D, Uzuner H, Baydemir C. Evaluation of immunochromatographic test for the detection of antibodies against Echinococcosis granulosus. Med Sci Monit. 2015;21:1219-22. doi: 10.12659/MSM.893155. PMID: 25921809; PMCID: PMC4427020.
28. Vola A, Manciulli T, De Silvestri A, Lissandrin R, Mariconti M, Siles-Lucas M, et al. Diagnostic Performances of Commercial ELISA, Indirect Hemagglutination, and Western Blot in Differentiation of Hepatic Echinococcal and Non-Echinococcal Lesions: A Retrospective Analysis of Data from a Single Referral Centre. Am J Trop Med Hyg. 2019;101(6):1345-1349. doi: 10.4269/ajtmh.19-0556. PMID: 31674293; PMCID: PMC6896875.
29. Park SJ, Han SS, Anvarov K, Khajibaev A, Choi MH, Hong ST. Prevalence of Serum IgG Antibodies to Cystic Echinococcus Antigen among Patients in an Uzbekistan Emergency Hospital. Korean J Parasitol. 2015;53(6):699-703. doi: 10.3347/kjp.2015.53.6.699. Epub 2015. PMID: 26797436; PMCID: PMC4725226.
30. Hernández-González A, Muro A, Barrera I, Ramos G, Orduña A, Siles-Lucas M. Usefulness of four different Echinococcus granulosus recombinant antigens for serodiagnosis of unilocular hydatid disease (UHD) and postsurgical follow-up of patients treated for UHD. Clin Vaccine Immunol. 2008;15(1):147-53. doi: 10.1128/CVI.00363-07. Epub 2007. PMID: 17989342; PMCID: PMC2223859.
31. Chirag S, Fomda BA, Khan A, Malik AA, Lone GN, Khan BA, et al. Detection of hydatid-specific antibodies in the serum and urine for the diagnosis of cystic echinococcosis in patients from the Kashmir Valley, India. J Helminthol. 2015;89(2):232-7. doi: 10.1017/S0022149X13000837. Epub 2014. PMID: 24429044.
32. Savardashtaki A, Sarkari B, Arianfar F, Mostafavi-Pour Z. Immunodiagnostic Value of Echinococcus Granulosus Recombinant B8/1 Subunit of Antigen B. Iran J Immunol. 2017;14(2):111-122. PMID: 28630382.
33. Jin Y, Anvarov K, Khajibaev A, Hong S, Hong ST. Serodiagnosis of echinococcosis by ELISA using cystic fluid from Uzbekistan sheep. Korean J Parasitol. 2013;51(3):313-7. doi: 10.3347/kjp.2013.51.3.313. Epub 2013. PMID: 23864742; PMCID: PMC3712105.
34. Iraqi W. Diagnostic value of semi-purified antigens of hydatid cyst fluid in human cystic echinococcosis. Acta Parasitol. 2016;61(1):144-50. doi: 10.1515/ap-2016-0019. PMID: 26751885.
35. Reiterová K, Auer H, Altintaś N, Yolasigmaz A. Evaluation of purified antigen fraction in the immunodiagnosis of cystic echinococcosis. Parasitol Res. 2014;113(8):2861-7. doi: 10.1007/s00436-014-3947-0. Epub 2014. PMID: 24828349.
36. Mamuti W, Yamasaki H, Sako Y, Nakaya K, Nakao M, Lightowlers MW, et al. Usefulness of hydatid cyst fluid of Echinococcus granulosus developed in mice with secondary infection for serodiagnosis of cystic Echinococcosis in humans. Clin Diagn Lab Immunol. 2002;9(3):573-6. doi: 10.1128/cdli.9.3.573-576.2002. PMID: 11986262; PMCID: PMC119995.
37. Bauomi IR, El-Amir AM, Fahmy AM, Zalat RS, Diab TM. Evaluation of purified 27.5 kDa protoscolex antigen-based ELISA for the detection of circulating antigens and antibodies in sheep and human hydatidosis. J Helminthol. 2015;89(5):577-83. doi: 10.1017/S0022149X14000479. Epub 2014. PMID: 25006882.
38. El-Shazly AM, Saad RM, Belal US, Sakr T, Zakae HA. Evaluation of ELISA and IHAT in serological diagnosis of proven cases of human hydatidosis. J Egypt Soc Parasitol. 2010;40(2):531-8. PMID: 21246959.
39. Santivañez SJ, Arias P, Portocarrero M, Rodriguez S, Gonzalez AE, Gilman RH, et al. Serological diagnosis of lung cystic hydatid disease using the synthetic p176 peptide. Clin Vaccine Immunol. 2012;19(6):944-7. doi: 10.1128/CVI.05540-11. Epub 2012. PMID: 22518012; PMCID: PMC3370447.
40. Eris FN, Akisu C, Aksoy U. Evaluation of two ELISA and two indirect hemagglutination tests for serodiagnosis of pulmonary hydatid disease. Korean J Parasitol. 2009;47(4):427-9. doi: 10.3347/kjp.2009.47.4.427. Epub 2009. PMID: 19967097; PMCID: PMC2788728.
41. Barbieri M, Fernández V, González G, Luaces VM, Nieto A. Diagnostic evaluation of a synthetic peptide derived from a novel antigen B subunit as related to other available peptides and native antigens used for serology of cystic hydatidosis. Parasite Immunol. 1998;20(2):51-61. doi: 10.1046/j.1365-3024.1998.00117.x. PMID: 9572048.
42. Shambesh MK, Craig PS, Gusbi AM, Echtuish EF, Wen H. Immunoblot evaluation of the 100 and 130 kDa antigens in camel hydatid cyst fluid for the serodiagnosis of human cystic echinococcosis in Libya. Trans R Soc Trop Med Hyg. 1995;89(3):276-

9. doi: 10.1016/0035-9203(95)90538-3. PMID: 7660432.
43. Sadjjadi SM, Abidi H, Sarkari B, Izadpanah A, Kazemian S. Evaluation of enzyme linked immunosorbent assay, utilizing native antigen B for serodiagnosis of human hydatidosis. Iran J Immunol. 2007;4(3):167-72. PMID: 17767016.
44. Shapiro SZ, Bahr GM, Hira PR. Analysis of host components in hydatid cyst fluid and immunoblot diagnosis of human Echinococcus granulosus infection. Ann Trop Med Parasitol. 1992;86(5):503-9. doi: 10.1080/00034983.1992.11812699. PMID: 1288431.
45. Wattal C, Malla N, Khan IA, Agarwal SC. Comparative evaluation of enzyme-linked immunosorbent assay for the diagnosis of pulmonary echinococcosis. J Clin Microbiol. 1986;24(1):41-6. doi: 10.1128/jcm.24.1.41-46.1986. PMID: 3522626; PMCID: PMC268828.
46. Barbieri M, Sterla S, Battistoni J, Nieto A. High performance latex reagent for hydatid serology using an Echinococcus granulosus lipoprotein antigen fraction purified from cyst fluid in one step. Int J Parasitol. 1993;23(5):565-72. doi: 10.1016/0020-7519(93)90161-q. PMID: 8225758.
47. Grimm F, Maly FE, Lü J, Llano R. Analysis of specific immunoglobulin G subclass antibodies for serological diagnosis of Echinococcosis by a standard enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol. 1998;5(5):613-6. doi: 10.1128/CDLI.5.5.613-616.1998. PMID: 9729525; PMCID: PMC95629.
48. Gadea I, Ayala G, Diago MT, Cuñat A, de Lomas JG. Immunological diagnosis of human cystic echinococcosis: utility of discriminant analysis applied to the enzyme-linked immunoelectrotransfer blot. Clin Diagn Lab Immunol. 1999;6(4):504-8. doi: 10.1128/CDLI.6.4.504-508.1999. PMID: 10391851; PMCID: PMC95716.
49. El-Ghareeb AS, Waked NM, Al-Feky HM. Clinical and parasitological studies on pulmonary and hepatic hydatid cysts in hospitalized children and adults. J Egypt Soc Parasitol. 2016;46(1):9-18. doi: 10.12816/0026145. PMID: 27363036.
50. Moghadam ZK, Ghaffarifar F, Khalilpour A, Abdul Aziz F, Saadatnia G, Noordin R. IgG4 detection of Echinococcus granulosus paramyosin is a useful diagnostic test for human hydatidosis. Clin Vaccine Immunol. 2013;20(4):501-5. doi: 10.1128/CVI.00019-13. Epub 2013. PMID: 23365208; PMCID: PMC3623400.
51. Shweiki HM, Bahr GM, Salama MS, Behbehani K, Hira PR. Analysis of the in vitro lymphoproliferative responses and antibody levels to the arc-5 antigen in patients with cystic hydatid disease. Ann Trop Med Parasitol. 1992;86(6):621-9. doi: 10.1080/00034983.1992.11812718. PMID: 1304704.
52. Ayadi A, Dutoit E, Sendid B, Camus D. Specific diagnostic antigens of Echinococcus granulosus detected by western blot. Parasite. 1995;2(2):119-23. doi: 10.1051/parasite/1995022119. PMID: 7582376.
53. Poretti D, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, et al. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am J Trop Med Hyg. 1999;60(2):193-8. doi: 10.4269/ajtmh.1999.60.193. PMID: 10072135.
54. Barbieri M, Severi MA, Pírez MI, Battistoni J, Nieto A. Use of specific antibody and circulating antigen serum levels in the hydatid immunodiagnosis of asymptomatic population. Int J Parasitol. 1994;24(7):937-42. doi: 10.1016/0020-7519(94)90157-0. PMID: 7883444.
55. Tawfeek GM, Elwakil HS, El-Hoseiny L, Thabet HS, Sarhan RM, Awad NS, et al. Comparative analysis of the diagnostic performance of crude sheep hydatid cyst fluid, purified antigen B and its subunit (12 Kda), assessed by ELISA, in the diagnosis of human cystic echinococcosis. Parasitol Res. 2011;108(2):371-6. doi: 10.1007/s00436-010-2074-9. Epub 2010. PMID: 20922427.
56. Manterola Delgado CG, Cuadra CA, Fonseca SF, Bustos ML, Hinostroza SJ. Utilidad de DD5 y ELISA-IgG como pruebas diagnósticas específicas en pacientes con hidatidosis hepática. Rev Chil Cir. 2003;55(1):25–9.
57. Flores LAA, Rodriguez HP. Estandarización de la prueba de ELISA para el inmunodiagnóstico de hidatidosis humana empleando antígenos de producción local. Gac Med Bol. 2006;29(1):5-10.
58. Manterola C, Cuadra A, Muñoz S, Sanhueza A, Bustos L, Vial M, et al. In a diagnostic test study the validity of three serodiagnostic test was compared in patients with liver echinococcosis. J Clin Epidemiol. 2005;58(4):401-6. doi: 10.1016/j.jclinepi.2004.10.009. PMID: 15862726.
59. Vacirca D, Perdicchio M, Campisi E, Delunardo F, Ortona E, Margutti P, et al. Favourable prognostic value of antibodies anti-HSP20 in patients with cystic echinococcosis: a differential immunoproteomic approach. Parasite Immunol. 2011;33(3):193-8. doi: 10.1111/j.1365-3024.2010.01264.x. PMID: 21306401.
60. Maglioco A, Gentile J, Barbery Venturi MS, Jensen O, Hernández C, Gertiser ML, et al. Detection of Echinococcus granulosus sensu lato infection by using extracts derived from a protoscoleces G1 cell line. Parasite Immunol. 2019;41(12):e12674. doi: 10.1111/pim.12674. Epub 2019 Oct 13. PMID: 31557338.)
61. Petrone L, Vanini V, Petruccioli E, Ettorre GM, Busi Rizzi E, Schininà V, et al. IL-4 specific-response in whole blood associates with human Cystic Echinococcosis and cyst activity. J Infect. 2015;70(3):299-306. doi: 10.1016/j.jinf.2014.10.009. Epub 2014 Oct 22. PMID: 25444973.

62. Vidor E, Piens MA, Abbas M, Petavy AF. Biochimie du liquide hydatique (Echinococcus granulosus). Influence de la localisation sur la perméabilité des kystes. Ann Parasitol Hum Comp. 1986;61(3):333-40. doi: 10.1051/parasite/1986613333. PMID: 3545031.

63. Zeghir-Bouteldja R, Polomé A, Bousbata S, Touil-Boukoffa C. Comparative proteome profiling of hydatid fluid from Algerian patients reveals cyst location-related variation in Echinococcus granulosus. Acta Trop. 2017;171:199-206. doi: 10.1016/j.actatropica.2017.03.034. Epub 2017. PMID: 28412048.