Diagnosis confirmation of human cystic echinococcosis by imagistic methods and immunoserological determinations

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Abstract. Cystic echinococcosis is a severe parasitic disease which affects both humans and animals. The disease has a considerable economic and social impact, because it has numerous complications leading to important disabilities and even death. The study was done during the period 2006-2010 and included 146 symptomatic persons from the endemic and no-endemic zones, with suggestive symptoms or with imagistic suspicion of hydatid disease. Abdominal ultrasound was done on all patients, which in some cases oriented us toward a diagnosis of unique or multiple hepatic, splenic, renal, and abdominal formations. Out of 146 persons tested by determining antiechinococcus IgG by using ELISA, 59% positively presented antiechinococcus IgG antibodies, 23% tested negatively for antiechinococcus IgG antibodies, and in 18% of the cases the result of the test was uncertain. Western Blot IgG Echinococcus determinations were done for 41% of the patients included in the study: 16% presented positive Western Blot serology for *E. granulosus*, 9% presented positive Western Blot serology for *E. granulosus* or *E. multilocularis*, and 16% presented negative serology, hydatid pathology being thereby excluded. The test Western Blot IgG Echinococcus confirmed the positive results and it was appreciated as being the most relevant test in various stages of cystic echinococcosis.

Keywords: Cystic echinococcosis; Imaging diagnosis; IgG antiechinococcus ELIS; Western Blot IgG Echinococcus.

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Introduction

Cystic echinococcosis is a severe parasitic disease which affects both humans and animals. The disease has a considerable economic and social impact, because it has numerous complications leading to important disabilities and even death.

The initial phase of primary infestation is usually asymptomatic. After a variable incubation period, when cysts grow, exerting pressure on adjacent tissues, or when complications appear, infestation becomes symptomatic.

Presumptive diagnosis of hydatidosis is established based on clinical or organic manifestations, sometimes accompanied by
allergic manifestations and based on the presence of hydatid with pseudo-tumoral aspect, of benign tumor, evidenced by imagistic techniques: ultrasound, CT scan, X-ray, MRI (Constantea and Ciobanca, 2007).

Sometimes, the lesion can be accidentally discovered by a routine X-ray or ultrasound examination.

The diagnostic methods are: epidemiological investigation, anamnesis and physical exams, completed by imagistic investigations: X-ray, ultrasound, CT scan, MRI, serological methods, such as ELISA, Western-Blot, and parasitological tests.

Serological examinations allow for an active discovery of persons suffering from hydatidosis in incipient phases, not detectable by imagistic methods, which helps establishing a precocious diagnosis for hydatidosis and it allows for instituting medical treatment and avoiding complicated forms or apparition of intra or post-op complications (Crețu, 2005).

In the absence of treatment, cysts grow in dimensions and finally determine the apparition of complications (the most frequent being the apparition of fistulas, abscesses, or ruptures).

**Purpose of study**

2. Classifying the imagistic criteria of cystic formations and their confirmation by serological methods, in order to establish differential diagnosis.
3. Evaluation of diagnostic tests: imagistic and serological, as screening and diagnostic methods of cystic echinococcosis.
4. Serological evaluation of imagistically inconclusive cystic formations.

Research in view of confirming the clinical and imagistic diagnosis of hydatid cyst by determining the IgG antiechinococcus using the Western Blot method.

5. Confirming the clinical and imagistic diagnosis by determining the IgG anti-echinococcus using the Western Blot method.

**Objectives of study**

1. Description of cystic echinococcosis distribution in positive persons, according to sex and original environment.
2. Precision of incidence of hydatid disease on age groups.
3. Evaluation by serological methods (ELISA) of the hydatid disease and actively finding the persons in precocious phases, detectable and not detectable by imagistic examinations.
4. Identifying cystic formations and defining criteria for hydatid or non-hydatid cyst by applying the methods of paraclinical diagnosis.
5. Comparative study of imagistic methods (ultrasound, X-ray, CT scan, MRI) with serological methods (ELISA, Western Blot).
6. Detecting IgG Echinococcus by the Western Blot method, for the ethiological confirmation of pseudo-tumoral aspects, imagistically inconclusive, with serological uncertainty by ELISA, in view if achieving a differential diagnosis.

**Material and method**

The study was done during the period 2006-2010 and included 146 symptomatic persons from the endemic and no-endemic zones, with suggestive symptoms or with imagistic suspicion of hydatid disease.

The persons included in the study presented a clinical or imagistic suspicion of hydatid cyst, uncomplicated or complicated with various localizations: hepatic, pulmonary, cerebral, splenic.

In accordance with the localization of the hydatid cyst, patients presented non-characteristic clinical manifestations (diffuse abdominal pain, pain in the right hypocondrium, asthenia, loss of appetite, weight loss), pulmonary manifestations (cough, dyspnoea, chest pain), neurological manifestations (focal manifestations, cephalea,
dizziness), allergic manifestations (urticaria, erythema, eosinophilia).

The diagnosis was established on clinical, imagistic, and laboratory criteria – usual serological tests (determination of IgG antiechinococcus by ELISA and Western Blot). There were persons introduced into the study who did not present any clinical manifestations, being diagnosed at a routine ultrasound checkup.

Human infection with *Echinococcus granulosus* determines production of IgG, IgM, IgA, and IgE class-specific circulating serum antibodies.

In our study we used the ELISA technique, with NovaTec kits for detecting IgG class-specific antibodies and the Western Blot technique.

**Abdominal ultrasound** was done on all patients introduced into the study. On some patients **chest X-ray** was done to exclude secondary pulmonary localization or suspicion of pulmonary hydatid cyst.

**CT scan** was done to confirm diagnosis in cases of tomographic or radiological uncertainty, to get a more minute description of the formation, to highlight the possible complications and detect other formations with smaller dimensions, which the first examinations could have overlooked.

**Serological tests (ELISA enzymatic immunodosage of IgG antiechinococcus)** were done on all patients introduced into the study.

We did **Western Blot determinations of IgG Echinococcus** on patients with diagnostic uncertainty, after doing the imagistic examinations and dosage of IgG antiechinococcus by using ELISA. Determinations were also done on patients that were imagistically and serologically confirmed for checking the methods (imagistic and dosages of IgG antiechinococcus by using ELISA).

**ELISA Technique** represents the qualitative immunoenzymatic determinations of IgG-class antibodies against *echinococcus* (Logar et al., 2008).

**The results** calculated in NovaTec units:

Value of sample absorbance x 10/marker sample = NTU
- Positive results: values ≥ 11
- Negative results: values ≤ 9 NovaTec units (NTU),
- Inconclusive results: values raging between 9 and 11 NTU
- Marker: 10 NovaTec units (NTU).

**Western Blot IgG Technique** is a qualitative immunoblot dosage with the following applications:

- test for serological confirmation of infections with *E. granulosus* and *E. multilocularis* done on human serum or plasma, previously tested by classic screening serological tests (IHA, IFA, ELISA);
- serological differentiation of *E. granulosus* from *E. multilocularis*. (Reiter-Owona et al., 2009).

**Interpretation** (Tappe et al., 2008)

1. Serodiagnosis of the *Echinococcus* genre
   - by determining molecular weight, the presence of bands of 7, 26-28 kDa is identified for each sample;
   - the bands of 26-28 kDa may present various aspects: there can be narrow bands, double narrow bands, one single wide band ranging between 26-28 kDa;
   - the presence on the strip of bands of 7 and/or bands of 26-28 kDa indicates the presence of *Echinococcus* specific IgG in the serum sample.

2. The differential diagnosis between *E. granulosus* and *E. multilocularis* is achieved by reading the specific intermediate bands between 7 and 28 kDa.

Bands that can be observed from 7 to 28 kDa:
- bands that are common to both species: 7, 12,15, 24, 26-28 kDa
- narrow bands that are commun to *E. multilocularis* only: 16, 17, 18, 20 kDa
- band common to *E. granulosus* only: a very wide band (with irregular shadow) between 16 and 18 kDa.

**Figure 1.** Typical models of identification of *Echinococcus* ECHWBG-a 25.08.2005

**Results and discussions**

*Distribution of symptomatic persons or with clinical and imagistic suspicion of hydatid cyst According to Gender*

Of the total number of cases included in the study (146 cases), with clinical or imagistic symptomatology of hydatid cyst, 53% were women and 47% men. It was found a greater incidence among women than in men.

**Figure 2.** Percentual distribution of cases with suspicion of hydatid cyst according to sex

Specialized literature mentions a greater incidence of hydatid disease in women, but there are studies that attest a greater incidence of the disease in men (Iacobiciu et al., 2002).

Worldwide we find a greater incidence in women as compared to men: in Tibet, the ratio between the cystic echinococcosis is of 2:1 in favor of the female gender, which can be probably explained by the tighter contact of women with dogs around their homes (Tiaoying et al., 2005).

A greater incidence was also exemplified in Turkey (Sevgili and Gökçen, 2008).

In our region, Coroiu and her team (Coroiu et al., 2007) observed an ascending evolution in the female gender both for Cluj district and in the whole North-Western region of Romania.

**Distribution of symptomatic persons or with clinical and imagistic suspicion of hydatid cyst According to their original environment**

Our study shows that the incidence of persons with suspicion of hydatid cyst was higher in the rural environment, considering that 54% of such suspicions were found in persons coming from the rural environment and 46% in persons coming from the urban environment.

**Figure 3.** Percentual distribution of cases with suspicion of hydatid cyst according to original environment

According to the data provided in literature, cystic echinococcosis is more frequent in the rural zones than in the urban zones by 50-60%, probably due to the intertwining of biological cycles of parasites (Jenkins et al., 2005; Craig and Larrieu, 2006).

The study reveals a higher incidence in the rural environment as compared to the urban environment, but the difference is not so significant as in the data provided by the specialized literature, which suggests that the differences in incidence of hydatid cyst according to the original environment, between the research done and the data reported in literature, might be due to the lack of information regarding the origin of hydatid...
cyst in adults or reflects another epidemiological context, related to the geographical area.

Distribution of symptomatic persons or with clinical and imagistic suspicion of hydatid cyst on age groups

According to a bibliographical analysis in the specialized literature, it was found that that hydatid infection is present in all age groups, except for children under 5 years old (Dopchiz et al., 2007).

In our study there were not included any persons under the age of 10.

In adults, clinical and imagistic suspicion of hydatid cyst was found in all age groups, with an incidence of 6% in youth aged 10-20, 13% in young adults aged 21-30, 15% in persons aged 31-40 ani, 14% in adults aged 41-50, being prevalent in persons aged 51-60, with a percentage of 21%, 20% in persons aged 61-70 and it has a low incidence in elderly persons over 70 (7% and 4%, respectively).

The decrease in number of cases was observed in age groups over 70, probably due to the withdrawal of such persons from active life.

![Figure 4. Percentual distribution of cases with suspicion of hydatid cyst on age groups](image)

The progressive increase of impact degree along with age shows an active transmission of the parasite, higher levels being seen in age groups that were exposed to the possibility of infestation for a longer period of time. It should also be mentioned that hydatid may have a slow evolution (for years), in many cases the disease being asymptomatic for a long period of time (until the apparition of complications or accidental discovery by routine medical tests).

Specialised literature specifies that the upper age limit should be 19 years, this is due to the slow evolution of hydatid, as a consequence of infestation since childhood.

In a study, Junie and the team (2002) pinpoint an incidence of 55% of cases of hydatid disease in child population, the most frequent being in the age groups 7-14 years.

The mean multiannual morbidity in children as reported by Coroiu and the team (2007) was of 5.5 cases in 100,000 inhabitants.

Evaluation of symptomatic persons with suspicion of hydatid cyst by imagistic methods

One of the main applications of ultrasound exploration continues to be detecting and characterizing hepatic masses. In practice, we found situations where a tumor is discovered by chance or as a result of an active "screening" conduct.

Although the anatomic data provided by ultrasound examination are numerous, it is necessary to correlate such data with the clinical and functional characteristics, and with the paraclinical laboratory tests (eosinophilia, basophilia, serological tests).

Ultrasound exploration is a clinically integrated technique, but more often we need to use punctures, biopsies, or other means of imagistic diagnosis.

We mention that a cystic lesion must be at least 0.8 cm in diameter to be detectable.

![Figure 5. Imagistic methods used to identify hydatid cyst](image)
Abdominal ultrasound was done on all patients, which in some cases oriented us toward a diagnosis of unique or multiple hepatic, splenic, renal, and abdominal formations. A person was subjected to supraclavicular ultrasound, which showed a cystic formation.

We mention that ultrasound was accompanied by X-ray for 48 patients, excluding secondary pulmonary localization or a suspicion if pulmonary hydatid cyst.

Abdominal CT scan was done on 23 patients to confirm the diagnosis in case of ultrasound uncertainty or to better describe the formation, to show possible complications and detect other smaller formations that ultrasound could omit.

In cases of suspicion of cerebral formation, cranial CT scan was accompanied by MRI for 6 persons.

The results of imagistic examination (ultrasound, chest X-ray, CT scan, MRI)

Following the imagistic investigations done, there were identified 75% hepatic hydatid cysts, 3% pulmonary hydatid cysts, 2% splenic hydatid cysts.

There were identified other formations as well: 8% simple biliary cysts, 2% hepatic cysts interpreted as hepatic polycystic disease, 2% renal cysts, 1% hepatic abscesses, 1% splenic abscesses, 1% splenic cysts, 1% pulmonary cysts, 1% supraclavicular cysts, giant peritoneal cysts, cerebral cysts.

In the case of patients who had cystic formations suggestive of a simple biliary cyst (12 patients), supplementary tests were needed (ELISA and Western Blot) to correctly establish the diagnosis, such tests excluding hydatid pathology.

In a patient who came in for a routine checkup it was identified a well-delimited hepatic tumor formation, which was initially (after ultrasound examination) suspected to be a hepatic hydatid cyst type Gharbi IV. A CT scan was performed to obtain a clearer morphological characterization, which raised the suspicion of teratoma. ELISA and Western Blot tests were negative. Considering that the hydatid character of the formation was excluded, it was recommended for the patient to have an aspiration puncture with fine needle, but he refused, due to the invasive character of the procedure.

A female patient presented a cystic formation occupying 2/3 of the upper left hemithorax. The CT aspect indicates pulmonary hydatid cyst. Supplementary investigations were made, namely IgG antiechinococcus by using ELISA and IgG antiechinococcus by using Western Blot, and both tests were negative. In the case of this patient, we checked ELISA method by doing it simultaneously in two laboratories. For a more precise diagnosis it would have been necessary to continue investigations, by invasive techniques, which (due to her altered general state) the patient refused.

Our research included a patient who had been operated two years ago for pulmonary neoplasm. The formation relapsed and imposed the differential diagnosis between disseminated pulmonary echinococcosis and possible posterobasal voluminous cyst fractioned in the pleura, left bronchopulmonary neoplasm with secondary pulmonary determinations, pulmonary metastasis originating in the ovaries. The patient was tested by craniocerebral CT scan, abdominal and pelvic CT scan to detect secondary determinations, both being negative. ELISA serological tests were positive, Western
blot was positive for *Echinococcus granulosus* or *Echinococcus multilocularis*. The investigations led the diagnosis in favor of disseminated pulmonary echinococcosis with posterobasal voluminous cyst fractioned in the pleura.

Figure 7. Pulmonary X-ray: Ovoid formation occupying 2/3 upper left hemithorax

In another patient who came in for a routine checkup there were found, following ultrasound examination, multiple peritoneal giant cyst formations. IgG ELISA and Western blot tests were negative. On CT scan there was found a kidney malformation (superumbilical kidney), which led to the diagnosis of urethral malformation, namely megahydroureter.

Figure 8. CT scan: cystic formation occupying 2/3 of the pulmonary area

Another patient introduced into the study, known for 10 years with a hydatid cyst formation localized in the spleen, was monitored imagistically and serologically. The results of investigations done 12 years before described a partially calcified cystic formation. At the moment of presentation, the patient was diagnosed with totally calcified splenic hydatid cyst. Antiechinococcus IgG determined by ELISA was negative. We mention that the patient was zootechnical engineer in a sheep farm (30,000 sheep).

Figure 9. Megahydroureter

Figure 10. CT reconstruction: totally calcified splenic hydatid cyst

A female patient presented on ultrasound cystic formations in the left liver lobe and a formation suggesting a hydatid cyst with renal localization. CT scan confirmed the presence of formations, oriented the two hepatic cyst formations to simple biliary cysts, infirmed the renal localization of the formation, indicating adrenal localization with suspicion of hemorrhagic pheochromocytoma, adrenal carcinoma, adrenal hydatid cyst. We mention that the serological diagnosis antiechinococcus IgG determined by ELISA was negative, so
ELISA test made it possible to exclude the diagnosis of hydatid cyst.

Another patient investigated had a cystic formation revealed by ultrasound, which was suspected to be a renal hydatid cyst. CT scan confirmed the presence of the cystic formation, but etiology remained uncertain. The serological diagnosis, which was positive, confirmed the hydatid etiology.

In the case of persons where ELISA was negative it was necessary either to repeat the test after 2-4 weeks on another sample, or to confirm the diagnosis by another diagnostic method. If the results of the second test appear in the uncertainty zone, the sample will be considered negative.

Out of 146 persons tested by determining antiechinococcus IgG by using ELISA, 59% positively presented antiechinococcus IgG antibodies, 23% tested negatively for antiechinococcus IgG antibodies, and in 18% of the cases the result of the test was uncertain.

Diagnosis confirmation of Hydatid Cyst by Determining Antiechinococcus IgG by Using ELISA

In the specialized literature, the immune response detected was associated with the localization, integrity, and vitality of the hydatid cyst (Zhang and MacManus, 2006). The cysts localized in the liver produced a more intense immune response than those with pulmonary localization. Irrespective of the localization, the tests done to detect antibodies were less sensitive with patients that had cysts with intact walls. The cysts localized in the brain and the spleen were associated with a lower serodiagnostic reactivity. The fissure or rupture of a cyst is followed by a sudden stimulation of the immune response.

In our study there were persons who had calcified cysts (imagistically revealed). The antibody level is very low in patients with intact cysts and especially in case of old, calcified cysts, therefore the results of ELISA antiechinococcus IgG tests were negative in these patients.

The results of ELISA antiechinococcus IgG tests should be considered in the context of all clinical, epidemiological, and imagistic (ultrasound and CT scan) findings (Sbihi et al., 2001). ELISA antiechinococcus IgG tests excluded the hydatid disease in persons with imagistic suspicion and made possible the

**Figure 11.** CT: Hepatic cyst formations and adrenal formation

**Figure 12.** Abdominal CT scan: renal hydatid cyst

**Figure 13.** Results obtained in antiechinococcus IgG serological tests done by using ELISA
differential diagnosis of hydatid cyst from other pathologies.

**Diagnosis confirmation of hydatid cyst by determination of Echinococcus IgG by using Western Blot**

The immunoblot technique used is a powerful test for confirmation and serological differential diagnosis between the two major relevant infections: cystic echinococcosis and alveolar (Liance et al., 2000).

Data interpretation was done in a clinical, epidemiological, and imagistic context, as well as serological, by using ELISA antiechinococcus IgG.

**Figure 14. Western Blot Strips**

The bands may have various appearances: thin, thickened, more or less colored. It is recommended that, before starting using the Western Blot method for antiechinococcus IgG dosage, one should use known serums.

After doing the imagistic investigations and the antiechinococcus IgG dosage by using ELISA, we did determinations using the Western Blot method for antiechinococcus IgG dosage in the patients with diagnostic uncertainty. There were also done determinations in imagistically and serologically confirmed patients for the verification of methods (imagistic and ELISA antiechinococcus IgG dosage).

**Figure 15. Results obtained in Echinococcus IgG serological tests done by using Western Blot**

Western Blot IgG *Echinococcus* determinations were done for 41% of the patients included in the study: 16% presented positive Western
Blot serology for *E. granulosus*, 9% presented positive Western Blot serology for *E. granulosus* or *E. multilocularis*, and 16% presented negative serology, hydatid pathology being thereby excluded.

The test Western Blot IgG Echinococcus confirmed the positive results and it was appreciated as being the most relevant test in various stages of cystic echinococcosis.

A negative result of Western Blot IgG Echinococcus does not exclude the possibility of infection.

This method cannot differentiate a co-infection with *E. multilocularis* and *E. granulosus* from an infection with *E. multilocularis* only, both of them will probably present the same model of *E. multilocularis*.

*E. multilocularis* was not diagnosed in Romania so far at any human hosts.

We mention that the study did not diagnose any case of alveolar echinococcosis. The method specified the probability of *Echinococcus granulosus* or *Echinococcus multilocularis* infection but no specific case of alveolar echinococcosis was identified.

*Echinococcus multilocularis* was not detected in Romania so far in any definitive host (Sikó Barabási and Cozma, 2008).

In most countries it already gained an endemic character and is still expanding with alarming rapidity, becoming an emerging disease.

A study from literature, where there were detected IgG antibodies in the serum by the Western Blot method, showed a high sensitivity for the detection of the echinococcal infection. The test allowed the distinction between infections with *E. granulosus* and *E. multilocularis* in 76% of the cases. It did not allow for discriminating between the active and the inactive forms of the hydatid disease. It was found cross-reactivity with neurocysticercosis, which rendered necessary for the serum to be retested with species-specific antigen (Liance et al., 2000).

**Conclusions**

1. The hydatid disease remains one of the present issues of public health.
2. Hydatid cyst may evolve asymptptomatically for years and be diagnosed by chance, during routine medical checkups.
3. Imagistic explorations are clinically integrated techniques in the hydatid disease, but is more often needed the puncture, biopsy, or other diagnostic methods beign used as well.
4. Although the information provided by the ultrasound examination are numerous – anatomically speaking – it is necessary to correlate such data with the clinical characteristics of the disease, as well as with the functional, lab test results (eosinophilia, basophilia, serological tests) etc.
5. There is a probability that the persons with imagistic uncertainty, but with positive serology, may have a hydatid cyst smaller than 0.8 cm, which requires the investigation by other methods (Western Blot, aspiration puncture by thin needle) or repeating the imagistic investigation of the patients after a certain period of time.
6. The results of ELISA antiechinococcus IgG tests should be considered in the context of all clinical, epidemiological, and imagistic (ultrasound and CT scan) findings.
7. The ELISA antiechinococcus IgG tests excluded the hydatid disease in persons with imagistic suspicion and made possible the differential diagnosis of hydatid cyst from other pathologies.
8. The antibody level is very low in patients with intact cysts, especially in those with old, calcified cysts.
9. Serological examinations allow an active detection of persons with hydatidosis in incipient phases, which are not detectable by imagistic examinations, which represents a premise of precocious diagnosis of hydatidosis.
10. The IgG Western Blot technique used is a powerful test for confirmation and serological differential diagnosis.
11. The remarkable specificity and sensitivity of the immunoblot technique led to its use as a test of confirmation and for the
serological diagnosis for differentiating between the two relevant major infections: cystic echinococcosis and alveolar echinococcosis.

12. Western Blot Echinococcus IgG determinations confirmed the diagnosis of hydatid disease in the patients with diagnostic uncertainty after the imagistic investigations and antiechinococcus IgG dosage by using ELISA.

13. The results of Western Blot antiechinococcus IgG tests should be considered in the context of all clinical, epidemiological, and imagistic (ultrasound and CT scan) findings.

14. The bands may have various appearances: thin, thickened, more or less colored. It is recommended that, before starting using the Western Blot method for antiechinococcus IgG dosage, one should use known serums.

15. A negative result of Western Blot IgG Echinococcus does not exclude the possibility of infection.

16. E. multilocularis was not diagnosed in Romania so far at any human hosts.

17. This method cannot differentiate a co-infection with E. multilocularis and E. granulosus from an infection with E. multilocularis only, both of them will probably present the same model of E. multilocularis.

References


