Echinococcosis
New Strategies for Serological and Molecular Diagnosis

Metin Korkmaz
Ege University, Faculty of Medicine
Department of Medical Parasitology

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Casoni Skin Test

An intradermal test, used to for diagnosis.

https://web.stanford.edu/class/humbio103/ParaSites2003/Echinococcus/Diagnostics%20&%20Treatment2.htm

Man's arm showing positive skin test for hydatid disease. Courtesy of CDC
Diagnosis of Echinococcosis
Primarily based on imaging techniques

- Imaging techniques
  - Ultrasonography
  - Radiology
  - CT scans and MRIs
- Serology
  - Antibody detection
  - Antigen detection
- Molecular
  - Mainly on the characterization of isolates
- Cyst aspiration or biopsy
Assays

Antibody Detection

- A specific antibody response, mainly of the IgG class
- Detectable IgA, IgM and IgE antibodies
- Almost all serological tests have been used
- Indirect hemagglutination test and enzyme-linked immunosorbent assay are the most widely used methods for detection of anti-Echinococcus antibodies.
- Using combinations of IHA and ELISA (or IFA) tests is recommended for serologic diagnosis
- A positive reaction is confirmed by immunoblot assay
Alveolar Echinococcosis

Em70 and Em90
Parasite Antigens
Nature, purity and quality

• Protoscolex
• Hydatid cyst fluid
  • Antigen B, a polymeric lipoprotein
  • Antigen 5
• Recombinant antigens
  • Lack of a standardised test system due to native antigens
  • Recombinant antigens may be an alternative source for standardization.
Assays

Antigen Detection

- Generally applicable for laboratory research purposes only
- Monitoring disease?
- There are no diagnostic tests in routine practise
New Perspectives

Simple to perform and not time consuming

- New and better tests for screening
- Practical, where conventional serology techniques are unavailable
- Standardization of antigen
- Simple, precise and reproducible diagnostic tests were required
Recombinant antigens

The 8 kDa subunit of AgB is the most studied antigen

- Antigen B8/1 and B8/2
  - Provide the highest diagnostic sensitivity and specificity.
- *Echinococcus* protoscolex calcium binding protein (rEPC1)
- Multi-Epitope Antigens (MEA-8, MEA-20, MEA-26, MEA-36, MEA-49, and MEA-52)*
- rEmAgB3 suggested as a promising biomarker for serological assessment of AE patients. It is highly correlated with worm viability**
- Single defined molecule may not properly diagnose echinococcosis
- No significant difference between recombinant and native antigens

Rapid Diagnostic Tests

- **VIRapid HYDATIDOSIS** *(Vircell, Salamanca, Spain)*
  - Based on purified antigen B and antigen 5

- **Echinococcus Dot Immunogold Filtration Assay** *(DIGFA, Unibiotest, Wuhan, China)*
  - Based on purified cyst fluid, protoscolex antigen, antigen B and antigen Em2 of *E. multilocularis*

- **ADAMU-CE** *(ICST, Saitama, Japan)*
  - Based on recombinant antigen B

- rEm18-ICT for alveolar echinococcosis.
(A) VIRapid HYDATIDOSIS test and its semiquantitative colorimetric scale. (B) ADAMU-CE test. (C) DIGFA test and its diagnostic and semiquantitative colorimetric interpretation; EgCF = E. granulosus Cyst Fluid antigen, EgP = E. granulosus Protoscolex antigen, EgB = E. granulosus antigen B, Em2 = E. multilocularis antigen 2.

PaGia is available to blood banks that use a gel centrifugation technology system.

High-density polystyrene beads suspended in a gel similar to those used in transfusion medicine and is read like a blood group test.
**Echinococcus granulosus PaGIA test**

- A commercial *E. granulosus* PaGIA test (DiaPro) which is based on recombinant antigens is underdevelopment.
- His tagged recombinant antigens: EgAg5, EgAgB8/2 and EgAgB8/1.
- Incubate 5 min, centrifuge 10 min
- Preliminary results showed acceptable sensitivity and specificity for detecting anti-*Echinococcus* antibodies.
Screening for Echinococcosis?
Why, whom, when to screen?

- Rationale: When detected and treated early the disease can be cured
- Ultrasound
- Serology
- US should be selected as the primary test in field studies*

Molecular approaches
Species identification, better understanding of pathogenesis

• Identification/discrimination of Echinococcus species in definitive and intermediate hosts.
• Better understanding of pathogenesis and disease
• Formalin-fixed paraffin-embedded liver tissue samples
• Multiplex PCR, which simultaneously using multiple specific primers in a single tube and detecting more than one target species, is an effective method for the identification of parasites.
Molecular approaches
Mitochondrial regions amplified and sequenced

- NADH dehydrogenase subunit 1 (nad1), 219 bp
- NADH dehydrogenase subunit 5 (nad5), 584 bp
- Cytochrome c oxidase subunit 1 (cox1), 471 bp
- Cytochrome c oxidase subunit 2 (cox2)
- 12S rRNA and Nad5 gene
- The 12S PCR was most sensitive of all tested*.
- A single PCR on the 12S gene proved to be very suitable for detection and specification of Taenia sp. and Echinococcus sp.
