



WORLD CONGRESS OF ECHINOCOCCOSIS
AND 1ST PERUVIAN CONGRESS
OF ECHINOCOCCOSIS



UNIVERSIDAD PERUANA
CAYETANO HEREDIA

CHALLENGES TO IMPROVE SEROLOGIC DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

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Serological tests

- Serological tests are useful for diagnosing hydatid infections in people who live in areas of endemicity because of their low cost and ease of performance.

Challenges to face with serological tests

- Improve the sensitivity
- Reduce cross-reaction with other parasites, especially in those areas where hydatid cyst is present along with other helminths such as *Taenia solium*.
- Develop tests to detect active infections or monitor the patient after treatment

Enzyme-Linked Immunoelctrotransfer Blot Test

TABLE 1. Sensitivity and specificity of the EITB assay for the diagnosis of human hydatid disease

EITB assay band (kDa)	Sensitivity (%) (hydatid; n = 71)	Cross-reactions (%)		Specificity (%) (Peruvian controls; n = 45)
		Cysticercosis (n = 30) ^a	Hymenolepiasis (n = 32)	
8	65	10	0	100
16	59	3	0	100
21	58	3	0	100
All three bands	58	10	0	100
Any or all bands	65	10	0	100

^a One patient with cysticercosis gave repeated indeterminate results and was excluded from the study.

Enzyme-Linked Immunoelctrotransfer Blot Test

TABLE 2. Comparative sensitivities and specificities of the EITB assay, ELISA, and DD5 test for the diagnosis of human hydatid disease

Parameter	No. of patients	No. (%) seropositive by:				
		EITB assay			ELISA	DD5 test
		8-kDa band	16-kDa band	21-kDa band		
Sensitivity						
Pulmonary	32	18 (56)	15 (47)	14 (44)	18 (56)	14 (44)
Hepatic	20	18 (80)	18 (80)	18 (80)	18 (80)	14 (70)
Multiple	9 ^a	6 (56)	5 (56)	4 (44)	5 (56)	4 (44)
Ovarian	2	1 (50)	0	0	0	0
Overall	63 ^b	41 (65)	37 (59)	35 (56)	40 (64)	30 (48)
Cross-reactions						
Cysticercosis	26 ^b	3 (12)	1 (4)	1 (4)	8 (31)	6 (23)
Hymenolepiasis	32	0	0	0	4 (13)	0
Specificity	45	45 (100)	45 (100)	45 (100)	6 (80)	45 (100)

^a Includes three patients with hepatic and pulmonary cysts; one patient with liver, peritoneal, and lung cysts; four patients with hepatic and peritoneal cysts; and one patient with hepatic, pulmonary, and subcutaneous cysts.

^b Eight serum specimens from patients with hydatidosis and four serum specimens from patients with cysticercosis were excluded because of an insufficient volume to run the DD5 assay.

Verastegui M 1992

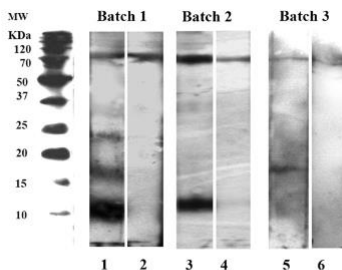


FIGURE 1. Representative Western blot profiles of different batches of AgB. Batches 1 and 2 showed a distinct band corresponding to the 8-/12-kDa subunit, whereas Batch 3 did not have the band. Molecular mass maker (10-250 kDa; Bio-Rad). Lanes 1, 3, and 5 show pooled positive serum samples from CE patients, and lanes 2, 4, and 6 show pooled healthy serum samples. MW = molecular mass.

Akbar Khalilpour et al 2014

Table 2. Diagnostic indices (%) of a sandwich ELISA used for the detection of anti-27.5 protoscolex antigen IgG and circulating protoscolex antigens in sheep and human sera of different studied groups.

Indices	Sheep		Human	
	Anti-27.5 kDa PA IgG	Circulating antigens	Anti-27.5 kDa PA IgG	Circulating antigens
Sensitivity	75.0	60.0	62.5	52.5
Specificity	80.0	88.0	66.7	75.0
Positive predictive value	79.0	83.3	55.6	58.3
Negative predictive value	76.2	68.8	72.7	70.3
Diagnostic efficacy	77.5	74.0	65.0	66.0

I.R. Bauomi et al 2014

Sources of obtaining antigen

- hydatid fluid
- Germinal layer
- Protoscolex (PSCs)
- Recombinant antigen

obtaining antibodies for antigen detection

- Protoscolex (membrane or E/S antigen)

Antigen detection in sera, urine, saliva

Obtaining PSC's from *E. granulosus*

- Naturally infected sheep
- slaughterhouses Lima and SAIS Túpac Amaru (Pachacayo - Huancayo)



Obtaining PSC's from *E. granulosus*

- **Enzymatic treatment of PSC's**

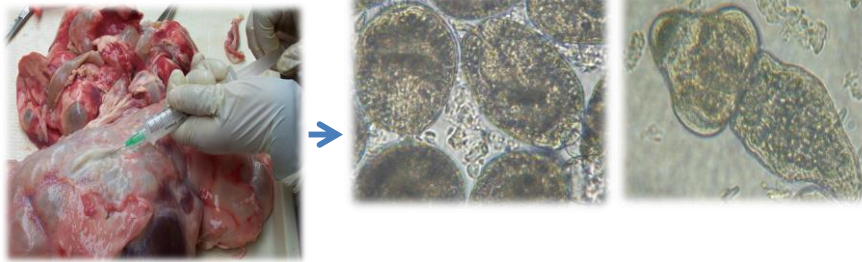
- AIF – Artificial intestinal fluid:

- Pork bile 1%,

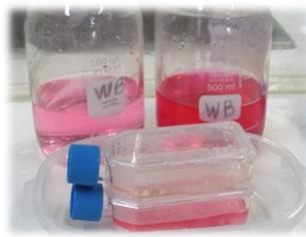
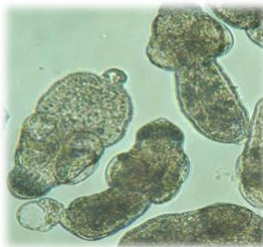
- Sodium bicarbonate 0.2%,

- Pancreatin 1%,

- Incubation : 1h ½



Obtaining E/S antigens from PSCs



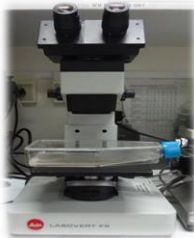
☐ Medio MEM
☐ Medio Ham F12
☐ Medio Ham F12
 (Suplementado)
 Glucosa (2%)
 Glutamina (0.5%)
 L-arginina (0.5%)

37°C , 95% de N₂ y 5% de CO₂

Antígeno recolectado c/24 h

Viabilidad y movilidad

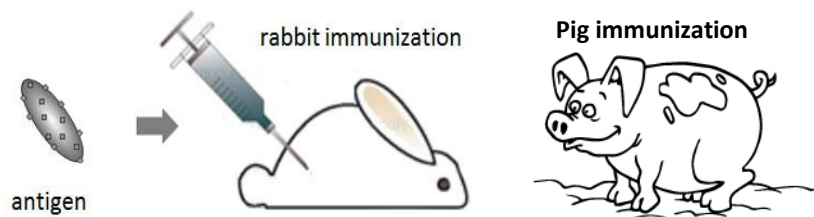
-70°C



Obtaining membrane antigens from PSC's

- Tritón x-114 (2%)
- Sodium deoxycholate(10%)

Antibodies against E/S and Membrane proteins of PSCs



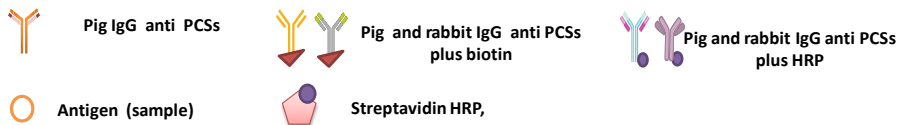
Detection antigen by ELISA

- In preliminary data using rabbit sera against E/S protein and membrane protein we detect antigen of hydatid cyst.
- Using a serum pool of patients positive for hydatid cyst, circulating antigen was detected, giving an absorbance twice as large as the pool of sera from negative or healthy individuals

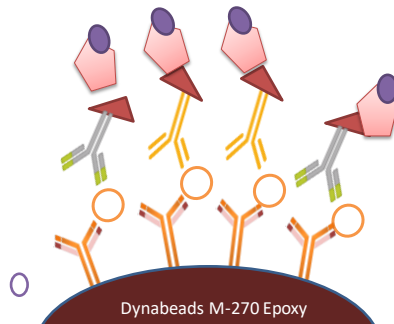
Preliminary results to detection antigen by ELISA in sera sample

Ratio of Sera sample	Capture antibody Pig- anti E/S- PCSs 1/25	Capture antibody Pig -anti E/S -PCSs 1/50
Pool positive/Pool negative	2.5	3
Conjugate -Rabbit sera against PM - PCSs 0.9ug/ml -HRP		

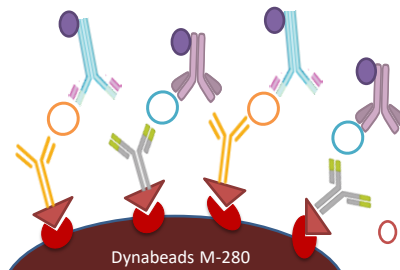
experimental design to be carried out



1. Design #1



2. Design #2



GRACIAS

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