

# Antibodies to *Echinococcus granulosus* Egg Antigens in Hydatid Patients: Characterization by Immunoblot and Diagnostic Value

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**Abstract** Cystic echinococcosis/hydatidosis, caused by *Echinococcus granulosus*, is a chronic zoonotic larval cestode infection in humans. Human hydatidosis is a public health parasitic disease that is cosmopolitan in its distribution. Several years may last between contamination with *E. granulosus* eggs and development of symptomatic cysts. Antigens expressed by embryos at early differentiation steps between the embryo and the young cyst may be interesting as markers of early infection or reinfection. This study investigates antibody response (IgG) to *E. granulosus* egg antigens by Immunoblot (IB) and ELISA in 20 hydatid patients surgically confirmed. We have also tested sera using protoscoleces and hydatid cyst fluid (HCF) from *E. granulosus*. The results show that hydatid patients reacted to eggs as well as protoscoleces and HCF. However, eggs share antigens in common with other life-cycle stages, but also support the notion that they may possess some unique stage-specific antigens determinants. The possible functional significance and the potential use of combined assessment of these antigens in IB with Ig G response in ELISA are discussed.

**Keywords:** cystic echinococcosis, hydatidosis, hydatid patients, echinococcus granulosus, hydatid cyst fluid, protoscoleces, eggs

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## 1. Introduction

Cystic echinococcosis (CE) or Hydatidosis, a parasitic disease due to *Echinococcus granulosus*, is an important zoonosis endemic in large parts of South America, East Africa, Australia, Central Europe, Central Asia and the Mediterranean littoral including North Africa [1]. In Tunisia, *E. granulosus* is geographically widespread and is the most significant zoonosis in the country, representing an important social and economic problem [2,3]. The annual average number of reported surgical cases of human hydatid disease, in 1998, was 1200 cases and the incidence rate is estimated to 15/100.000 inhabitants [4]. Ultrasonography is the imaging technique of choice for the diagnosis of CE. This technique is usually complemented or validated by computed tomography (CT) and/or magnetic resonance imaging (MRI) scans. Cysts can be incidentally discovered by radiography. Specific antibodies are detected by different serological tests and can support diagnosis. CE is often expensive and complicated to treat, sometimes requiring extensive surgery and/or prolonged drug therapy [5,6]. The development of cyst to a sizable level is a slow process. Several years may last between contamination with *E. granulosus* eggs and development of symptomatic cysts.

In humans, CE is contracted by ingestion of tapeworm *E. granulosus* eggs released from dogs. The hatched oncospheres penetrate the intestine, enter the blood stream and subsequently develop into the hydatid cysts in various organs, mainly liver and lungs. Hydatid cyst fertility is featured by the development of protoscoleces inside the cyst. The dissemination of protoscoleces from the primary site may lead to multiple secondary hydatid cysts, as reported by many authors. In Tunisia, 93.3% of hydatid cysts removed from patients was categorized as fertile [7]. The cyst fertility was independent of its site or its size. The same result was recorded in the child [8]. CE in slaughtered domestic ruminants and in the wild carnivores from Tunisia was published [9,10]. In the same context, the molecular identification of *E. granulosus* was reported [11].

Hydatid cyst fluid (HCF) is the most studied material compared to other *E. granulosus* extracts and is generally used as an antigen source for serodiagnostic purposes [12]. Two major parasite antigens, designated as antigen 5 (Ag 5) and antigen B (Ag B) were characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting (IB) [12,13]. Ag 5, a thermolabile lipoprotein, produces two subunits of about 53 and 64 kDa molecular weight respectively in SDS-PAGE under non-reducing conditions [13,14]. and two subunits of 38 and 24 kDa in reducing conditions [14]. Ag

B, a thermostable lipoprotein, presents in SDS-PAGE, three sub-units having 8/12, 16 and 24 kDa in both reducing and non-reducing conditions [14]. Detection of antibodies to hydatid Ag 5, is a sensitive and specific serodiagnostic test of human hydatidosis although antibodies cross-reactive with Ag 5 were also reported in other helminthic infections [15,16]. In addition, Ag B of 8/12 kDa was reported to be a putative diagnostic molecule using IB and immunoprecipitation analysis [17,18,19,20,21]. Modulation of human immune response by Ag B and its possible role in evading host defenses was reported [22].

In contrast to HCF, scarce studies have been devoted to analyze antigenic composition of oncospheres and investigate antibody response of humans with either symptomatic or asymptomatic *E. granulosus* infection. Since oncospheres represent the first premetacystode stage of *E. granulosus* larval development which enters into contacts with the host immune system, they may induce an antioncosphere immune response to antigens expressed at this early larval stage. Antibodies to oncospheres which have killing activity have been reported in experimentally infected lambs with *Teania hydatigena* and *Teania ovis* [23,24]. In addition, only little information is available on human antibody response to *E. granulosus* oncosphere antigens in hydatid patients. All tests developed so far, have been evaluated as tools for the diagnosis of fully developed cystic disease. No test has been validated as a test of human infection and especially of early infection. Antigens recognized by the immune system of the infected host at the very early steps of infection may be different from those recognized at later steps, and may have variable expression in time. Antigens expressed by embryos at early differentiation steps between the embryo and the young cyst may be interesting as markers of early infection or reinfection. Anti oncospherical antibodies were detected by immunofluorescence in people living in the hyperendemic area of Turkana in Kenya [25]. Moreover, these sera had killing activity of taeniid oncospheres mediated by complement [26]. Diagnostic value of this in vitro killing of taeniid was not evaluated. Furthermore, EA were used as vaccine in lambs [27,28], but no studies has been undertaken so far to analyse seroreactivity of hydatid patients with *E. granulosus* eggs.

In the present study, we analysed by IB and ELISA, the immunoreactivity with *E. granulosus* EA of sera from patients with CE and controls. The sensitivity, specificity and the predictive values of tests, using EA, were determined and compared to those using HCF and PA. In addition, hyperimmune rabbit antisera to EA, PA or HCF were used to investigate cross antigenicity between these three antigenic preparations.

## 2. Materials and Methods

### 2.1. Antigens

#### 2.1.1. Eggs

Gravid *E. granulosus* worms were collected from naturally infected stray dogs, caught in central and north eastern regions in Tunisia heavily infested with this parasite. Dogs were euthanized by injecting T 61 (Hoechst). Intestines were opened and inspected for *E.*

*granulosus* worms. Only dogs heavily infested (> 20.000 worms / intestine) were selected for egg collection. Intestines were soaked in warm normal saline (0.9% NaCl W/V), stirred intermittently until all worms are released. Worms were species identified and *E. granulosus* worms were sorted, washed ten times by saline with repeated decantations of excess liquid. *E. granulosus* worms were then incubated, for 2 hours at 37°C, with continuous stirring in a solution of 1% pepsin and 1% HCl in saline (pH 2) (Artificial Gastric Fluid (AGF)). Extraction of eggs was performed as in [25]. Pepsinized material was centrifuged at 1000 x g for 5 min, the pellet was resuspended in 1 ml saline, layered onto 1 ml neat Percoll (Pharmacia) and left for 5 mn to allow dense debris and grit to sediment, then the upper layer of eggs and pepsinized tissue was transferred into 20 ml of 0.15 M phosphate-buffered saline (PBS), pH 7.2, washed extensively and finally resuspended in PBS (V/V) containing 20 mM iodoacetamide, 2mM ethylene diamine tetra-acetic acid (EDTA), 1 mM phenyl methyl sulphonyl fluoride (PMSF), 2µg/ml pepstatin A and 10 µl/ml-aptotinin (All enzyme-inhibitors were from Sigma). They were sonicated on ice by repeated 10 sec strokes followed by 5 sec off until no intact eggs were seen microscopically. The eggs required prolonged sonication for complete rupture (15 mn). The whole egg sonicate (EA) were aliquoted and stored at -80°C.

#### 2.1.2. Protoscoleces

Protoscoleces were obtained from the pellet of centrifuged fertile hydatid cysts collected from sheep and by repeated washes of the proligere membrane of the cyst. They were washed three times in PBS, pH 7.2 and resuspended in the same buffer containing enzyme-inhibitors as described above. The protoscoleces suspension was sonicated on ice for 10 sec on and 5 sec off (3 mn) until they are disrupted. Whole sonicated protoscoleces antigens (PA) was separated into aliquots and stored at -80°C.

#### 2.1.3. HCF

Fertile hydatid cysts were obtained from infected sheep slaughtered in a regional abattoir. HCF was aspirated aseptically from liver cysts, clarified by centrifugation at 2000 g for 30 mn, at +4°C, separated into aliquots and stored at -80°C.

The protein content of EA, HCF and PA were estimated [29].

## 2.2. Rabbit Hyperimmune Antisera to EA, PA or HCF

Hyperimmune anti sera to EA, PA or HCF were raised in rabbits by three subcutaneous injections of 200 µg of the relevant antigen at three weeks intervals. The first dose was emulsified in Freund's complete adjuvant, the second dose was emulsified in incomplete Freund's adjuvant and the third dose was diluted in saline (0.9% W/V). Ten days after the last immunising dose, the rabbits were bled and sera were collected. Rabbits were also bled before immunisation (control preimmune sera).

## 2.3. Human Sera

Blood samples were collected from 20 Tunisian patients with surgically proven hepatic CE, (age: 35-47

years). A control panel of sera was obtained from 42 individuals infested with various non-*Echinococcus* parasites or viruses. This panel was composed as follows : sera from 8 individuals with ankylostomiasis (stools contained eggs of *Ankylostoma*), sera from 3 persons with schistosomiasis (stools contained eggs of *Shistosoma mansoni*), 6 sera from individuals of ascariasis (stools contained eggs of *Ascaris*), 3 sera from individuals of hymenolepiasis (*Hymenolepis nana* eggs were detected in stools), 10 sera from chronic carriers of Hepatitis B virus, 10 sera from children with acute Visceral Leishmaniasis due to *Leishmania infantum*. 13 sera were also collected from Tunisian healthy persons as negative controls.

## 2.4. Monoclonal Antibody

A monoclonal antibody (moAb) designated EG 02 154/12 specific for a proteic epitope of *E. granulosus* Ag 5 was used, to identify this antigen in EA and PA [15].

## 2.5. Electrophoresis and IB

EA, PA and HCF were separated under non-reducing conditions on SDS-PAGE using 13% mini-gel [30]. Total proteins were stained with Coomassie brilliant blue. EA, PA and HCF were resolved by SDS-PAGE, then electrophoretically transferred at 50 V for 1 hour 30 mn onto nitrocellulose paper (Millipore) in a cooled Biorad Transblot Cell with plate electrodes [31]. Non-binding sites on nitrocellulose were blocked, overnight at +4°C, using 0.15 M PBS, pH 7.2 containing 0.1% Tween 20 in 5% milk. Following blocking, the nitrocellulose was probed with sera diluted 1:100 in the blocking buffer from CE cases, from non-*Echinococcus* infections or from normal sera. In some experiments, blots were incubated with the rabbit hyperimmune antisera (diluted 1:200) or with the moAb to Ag 5 (diluted 1:500). All subsequent steps were performed at room temperature. Strips were incubated in diluted sera for 1.5 hour on rocking platform. Strips were then washed with 4 x 10 mn changes of PBS containing 0.1 % Tween 20. Horseradish peroxidase conjugated goat anti-human Ig G ( $\gamma$ -chain specific) (Sigma) or rabbit anti-mouse Ig G (Jackson Immuno-Research), diluted 1:1000 or pig anti-rabbit Immunoglobulins (Dako) diluted to 1:2000 in the blocking buffer, was added and incubated for 1 hour. The strips were washed as above. Peroxidase reactivity was visualized with diaminobenzidine (Sigma) plus 0.01% H<sub>2</sub>O<sub>2</sub> 30%.

## 2.6. ELISA

Polystyrene microtiter plates (Costar) were coated with 100  $\mu$ l per well of the antigen preparations using an antigenic concentration of 10  $\mu$ g protein/ml for EA and PA and 5  $\mu$ g of protein/ml for HCF, diluted in 0.05 M bicarbonate-carbonate buffer, pH 9.6 and incubated overnight at +4°C. Wells were washed in PBS plus 0.1% Tween 20 and blocked with PBS containing 0.3% gelatin (Sigma) (blocking buffer) for 1 hour. Human serum samples were optimally diluted in 1: 200 in duplicate, placed directly into blocking buffer, incubated at room temperature for 2 hours, and the plate was then washed five times with PBS plus 0.1% Tween 20. A 100  $\mu$ l of Horseradish peroxidase conjugated goat anti-human Ig G

( $\gamma$ -chain specific) (Sigma), diluted 1: 2000 in the blocking buffer, was added and incubated for 1 hour. After the wells were washed as above, the substrate O-phenylenediamine (Sigma), diluted in 0.1 M citrate buffer pH 5.0 plus H<sub>2</sub>O<sub>2</sub>, was added (200  $\mu$ l per well). The reaction was stopped with 50  $\mu$ l of a solution of 1 M NaOH. The absorbance values were measured at 492 nm using a spectrophotometer (Labsystem Multiscan).

**Interpretation of ELISA results:** The cut-off level in the quantitative ELISA was determined by the mean value of absorbance obtained with sera from the 13 healthy controls and the 42 non-*Echinococcus* infected individuals, plus 2 standard deviations (SD).

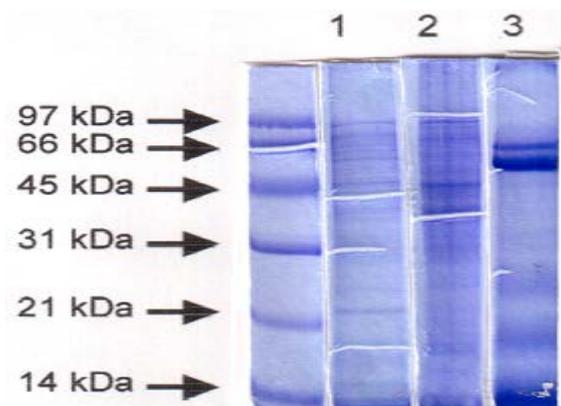
$$\text{Sensitivity} = \frac{\text{Number of true positive results}}{\left[ \begin{array}{l} \text{Number of true positive} \\ + \text{false negative results} \end{array} \right]} \times 100$$

$$\text{Specificity} = \frac{\text{Number of true negative results}}{\left[ \begin{array}{l} \text{Number of true negative} \\ + \text{false positive results} \end{array} \right]} \times 100$$

## 3. Results

### 3.1. Comparative Analysis of EA, PA and HCF on SDS-PAGE

Coomassie blue staining of SDS-PAGE of EA, PA and HCF showed a complex profile which appeared unique to each preparation. As expected, HCF was composed of two main components. Ag 5 which appeared as a doublet of 53-64 kDa and Ag B which appeared as bands of multiple of 8 kDa (8, 16, 24 and 32 kDa). EA and PA showed at least 20 thin bands mainly between 30 and 100 kDa as compared to HCF. No prominent band equivalent to Ag B or Ag 5 could be detected within these preparations. A 25 kDa protein appeared specific to egg extracts (Figure 1).

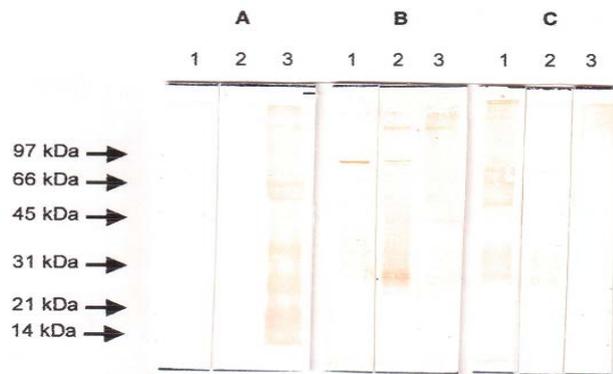


**Figure 1.** 13% SDS-PAGE pattern of *E. granulosus* EA, PA and HCF in non-reducing conditions. The position of the molecular weight markers is displayed on the left (Lane 1: EA, Lane 2: PA, Lane 3: HCF)

### 3.2. Comparative Analysis of EA, PA and HCF by IB Using Rabbit Hyperimmune Antisera

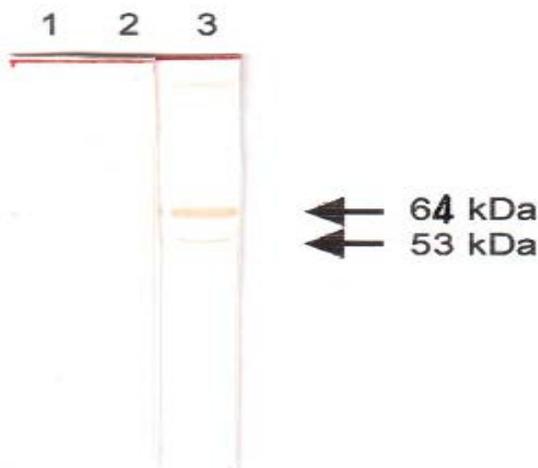
In order to characterize the immunogenic components of the three antigenic preparations (ie : EA, PA and HCF) and check for any antigenic cross reactivity between them, comparative IB analysis was performed using hyperimmune

antisera raised in rabbits against EA, PA and HCF respectively. Each antiserum was previously tested by ELISA and found to be strongly reactive with the homologous antigen (Absorbance values were higher than 2 at 1:500 dilutions, data not shown). Figure 2 shows the patterns of IB reactivity with EA, PA and HCF of the three antisera.



**Figure 2.** IB reactivity of hyperimmune rabbit antisera to HCF, PA or EA with HCF, PA or EA. The position of the molecular weight markers is displayed on the left. (A: Hyperimmune rabbit antisera to HCF, B: Hyperimmune rabbit antisera to PA, C: Hyperimmune rabbit antisera to EA) (Lane 1: EA, Lane 2: PA., Lane 3: HCF)

Rabbit antiserum to HCF, strongly stained the two main antigenic components of HCF (Ag B and Ag 5) but was completely unreactive with PA or EA. Rabbit antiserum to PA revealed mainly a 24 kDa component of PA but did not stain any of the Ag B component in HCF showing that the 24 kDa antigen in PA is antigenically not related to Ag B. Rabbit antiserum to PA did not stain any 53 or 64 kDa neither in PA nor in EA. However, a faint 53 kDa antigen could be detected in HCF. Finally, rabbit antiserum to EA strongly reacted with several bands of the homologous immunogen mainly 24 and 32 kDa components (which were also detected in PA) and a 50 kDa component. However, there was no reactivity with the Ag B and Ag 5 of HCF. All these data suggest that cross antigenicity between EA, PA and HCF is rather limited and that the main components of HCF (Ag 5 and Ag B) were undetectable in eggs and protoscolecis extracts. The absence of Ag 5 in egg and protoscolecis extracts was further confirmed by IB using the moAb to Ag 5 (Figure 3).

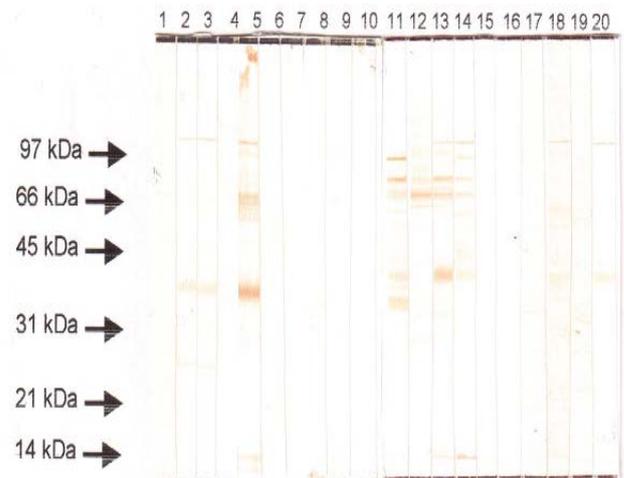


**Figure 3.** IB reactivity of moAb specific to Ag 5 with EA, PA and HCF. The molecular weight of the two subunits revealed, is displayed on the right (Lane 1: EA, Lane 2: PA, Lane 3: HCF)

### 3.3. Analysis of EA, PA and HCF by IB Using Sera from Hydatid Patients and Controls

#### 3.3.1. Reactivity to EA

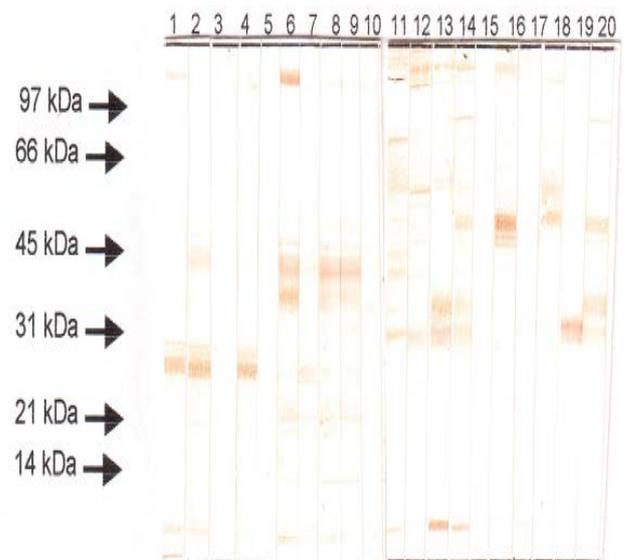
*E. granulosus* egg sonicate separated by SDS-PAGE was screened by IB with sera from hydatid patients and controls. Only nine sera from hydatid patients (45%) were reactive. Seven antigens of 24, 32, 40, 53, 64, 70 and 120 kDa were variably revealed (8 distinct patterns). The IB profiles generated by the 9 reactive sera differed from one serum to another (Figure 4).



**Figure 4.** IB reactivity to EA of 20 hydatid patient sera. The position of the molecular weight markers is displayed on the left

#### 3.3.2. Reactivity to PA

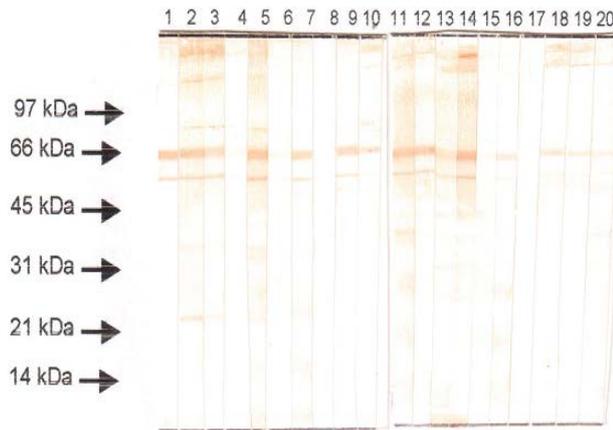
Fifteen out of 20 sera (75%) from patients with CE reacted with several PA of 8 kDa, 24 kDa (doublet), 30 kDa, 32 kDa (doublet), 34 kDa, 45 kDa (triplet) and 50 kDa molecular weight respectively. The predominant antigens were 30 kDa, 32 kDa and 45 kDa. Antibody reactivity of each individual serum was highly variable and 11 different patterns were generated by the 15 sera (Figure 5).



**Figure 5.** IB reactivity to PA of 20 hydatid patient sera. The position of the molecular weight markers is displayed on the left

### 3.3.3. Reactivity to HCF

Eighteen out of 20 (90%) sera from patients with CE reacted by IB with HCF antigens. As expected, the two major antigens detected were Ag5 (64/53 kDa) and AgB subunits (8, 16, 24 and 32 kDa). Almost all sera (18/20, 90%) reacted with the two subunits of Ag 5 but only 8 sera (40%) reacted with the 8 and 16 kDa subunits of Ag B. The 24 and 32 kDa subunits of Ag B were detected by only 11 (55%) and 3 sera (15%) respectively. Two sera were totally unreactive with HCF, as well as with EA and PA (Figure 6).



**Figure 6.** IB reactivity to HCF antigens of 20 hydatid patient sera. The position of the molecular weight markers is displayed on the left

Control sera from 42 patients with pathological conditions other than CE and from 13 normal individuals were all unreactive by IB with EA, PA and HCF antigens, except one serum from a patient with chronic carrier of Hepatitis B virus which reacted with the three antigenic preparations. The reactivity of this serum was almost identical to there generated by some CE sera (ie : Ag 5 and Ag B in HCF, antigens 32, 53 and 64 kDa in EA, antigens 30, 32 kDa (doublet) and 45 kDa (triplet) in PA).

When the patterns of Ig G reactivity against the three preparations were compared together, it appeared that three antigens of respectively 40, 70 and 120 kDa molecular weight respectively were detected only in egg extracts and have no equivalent bands in PA and HCF.

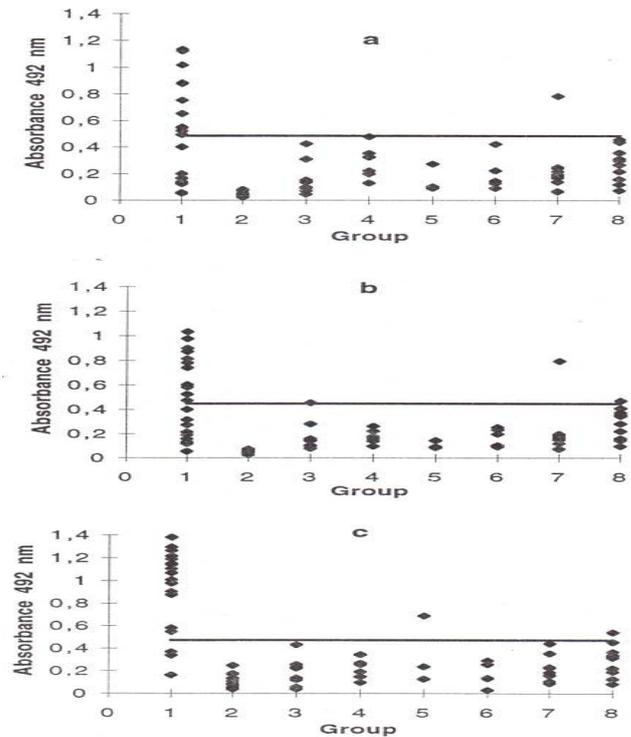
### 3.3.4. Reactivity of Sera from Hydatid Patients and Controls against EA, PA and HCF by ELISA

Sera from hydatid patients and controls were tested by ELISA against EA, PA and HCF. The reactivity of CE sera was consistently higher against EA, PA and HCF than of control sera (Table 1).

**Table 1. Serological response to EA, PA and HCF in ELISA**

Antigen used	Absorbance 492 nm ± Standard deviation		
	Patients with CE (n=20)	Non CE persons (n=42)	Healthy controls (n=13)
EA	0.499 ± 0.367	0.226 ± 0.148	0.045 ± 0.016
PA	0.507 ± 0.318	0.206 ± 0.139	0.047 ± 0.017
HCF	0.920 ± 0.345	0.230 ± 0.140	0.108 ± 0.060

The distribution of individual results obtained with 20 hydatid, 13 healthy persons and 42 non-*E. granulosus* sera, are presented in Figure 7 for EA, PA and HCF.



**Figure 7.** Distribution of individual results obtained in ELISA to EA (a), PA (b) and HCF (c) in 20 sera from patients with CE, 13 sera from healthy controls and 42 sera from subjects with other diseases. Mean absorbance value of the 55 sera controls plus 2 SD was taken as cut off level for positivity (Horizontal line). (Group 1: Patients with CE (n=20); Group 2: Healthy controls (n=13); Group 3: Individuals with ancylostomiasis (n=8); Group 4: Individuals with ascariasis (n=6); Group 5: Persons with hymenolepiasis (n=3); Group 6: Persons with shistosomiasis (n=5); Group 7: Individuals with viral hepatitis B (n=10); Group 8: Individuals with visceral leishmaniasis (n=10))

The specificity and the sensitivity of ELISA using either of the three antigens, are showed in Table 2. The specificity of ELISA using EA was very high (98%) but the sensitivity was low (55%). However, HCF gave the best combination of specificity (96%) and sensitivity (85%) as compared to EA and PA.

**Table 2. Validity parameters of ELISA using EA, PA and HCF**

	Antigen used		
	EA	PA	HCF
	Cut-off (0.48)	Cut-off (0.47)	Cut-off (0.44)
<b>Sensibility</b>	55%	55%	85%
<b>Specificity</b>	98%	94%	96%

In order to determine the most immunogenic antigens extracted from eggs, protoscoleces and HCF, we compared individual absorbance for each serum tested in ELISA with pattern obtained in IB, since the same profile gave different absorbance value (ie : 7, 10). We found that three (9, 10, 19) out of the eleven sera negative in IB were positive in ELISA using EA. This positivity was remarked with three (3, 5, 10) out of the five sera which were negative in IB using PA. However, the two patients negative in IB against HCF were also negative in ELISA (Data not shown).

## 4. Discussion

The present study compared the patterns of sera immunoreactivity from CE patients with antigenic extracts

of eggs, protoscoleces and HCF as tested by IB and ELISA. HCF has been repeatedly shown by several studies to be a suitable material for serodiagnosis of human CE. However, antigenic fractions in *E. granulosus* eggs were not reported in human hydatidosis.

Eggs were collected from naturally infected stray dogs which were found in autopsy to be heavily infested by *E. granulosus* worms. Although the latter were sorted to ascertain species homogeneity, we can not exclude a small contamination of the egg preparation by other taeniid eggs coinfecting these dogs.

The IB analysis using rabbit hyperimmune anisera to EA, PA and HCF showed that the cross reactivity between the three preparations were rather limited. Moreover, Ag 5 and Ag B, the major components of HCF, appeared absent in EA and PA. This was further confirmed using the moAb to Ag 5.

Previous studies have shown that protoscoleces contain Ag 5 and Ag B as revealed by Immunofluorescence analysis [32]. Our data suggest that these antigens are not extractable by homogenization and are not detectable in the extracts used in our study. The absence of Ag 5 and Ag B in eggs may explain the lower absorbance obtained with hydatid patients sera and the lower performance of the test using EA. HCF is still the best antigen for diagnostic purposes, as confirmed by others [33].

Our results showed that about half the sera from CE patients react with several antigens expressed by *E. granulosus* eggs. This low sensitivity could be explained by the disappearance of antibodies to EA in these patients who have surgically proven CE. In lambs infected with *T. hydatigena* or *T. ovis*, a rapid rise and a relatively rapid fall in antibody activity against eggs were demonstrated [23]. The rapid migration of the eggs in the intestine (30-120 mm) and their differentiation into cysts, between 1-15 days post-fixation of the embryos, must be considered [34,35,36,37]. A poor antigenicity of eggs could also account for the result mentioned above.

The reactivity detected against EA in some CE patients could suggest a boost of B memory cells by a recent contact with *E. granulosus* eggs. Cross-reactivity between EA and PA or HCF can not be ruled out although we consider it as unlikely taking into account the data obtained with rabbit anti sera to EA, PA and HCF. It is also important to mention that the pattern of reactivity with EA was heterogen. This heterogeneity may be attributed to the clinical history of the patients considered (closer contact with *E. granulosus* and age of infection). Existence of different *E. granulosus* genotypes infecting humans may also explain this response [38].

The low sensitivity obtained in ELISA, using EA, could be explained by the absence of Ag B and Ag 5 in this antigenic preparation (55%). In contrast, a high specificity was noted (98%). However, HCF showed good specificity (96%) associated to high sensitivity (85%) in comparison to EA in ELISA. These results demonstrate that HCF antigens have a good diagnostic value in hydatidosis.

The results obtained by IB using egg, protoscoleces and HCF antigens showed that some antigens of 40, 70 and 120 kDa are restricted to eggs. Precise role of these antigens in patients is difficult to ascertain since it is impossible to determine the age of hydatid cyst. Further work should concentrate on a better evaluation of the antigens specificity present in eggs. Specific antigen(s) in

eggs could help in determining recently acquired infection. For this reason, we have developed a model of *E. granulosus* infection in lamb (Paper under preparation).

Hydatid patient sera were also reactive to PA. However, a diversity of pattern was also obtained. This may be attributed to the age of infection, the disease character in the patients, the number of cysts and its spontaneous rupture. Protoscoleces contained many immunogenic antigens in hydatid patients. Nevertheless, antigens of 30, 45 and 50 kDa seem to be specific and could indicate fertilized or a ruptured cyst. Ag 5 was not detected neither with moAb nor with sera from hydatid patients. However, Ag 5 was detected, by indirect immunofluorescence, in the internal area of the germinal membrane, the parenchyme and the excretory system of the protoscoleces [17,32]. The authors proposed that Ag 5 is excreted by the larvae into the hydatid cyst where it may accumulate and become easily detectable by IB while in the protoscoleces, this antigen was difficult to detect. However, the subunits of 8, 24 and 32 kDa detected in PA might represent the Ag B, since they have the same molecular weight. Indeed, some authors have reported that Ag B is released by the protoscoleces into the cystic cavity [17]. The antigens 24 and 32 kDa revealed in eggs, by hydatid patient sera, might be the same as that described in protoscoleces when we consider their molecular weight.

The results obtained by ELISA demonstrated that HCF was the most immunogenic antigen in hydatid patients. We have noted a sensitivity about 85% in ELISA and 90% in IB. Such results may be related to the stage of the infection or to the immune system of the patients. Other reasons could be attributed probably to the permeability of the hydatid cyst wall which may lead to the permanent release in the circulation, of the hydatid antigens, which in turn may have resulted in durable humoral immune response. In contrast, the protoscoleces appear to be well protected from the host immune by its proligere capsule. The transient stage of eggs in the *E. granulosus* life and the robust protection conferred by the embryophore could also explain the result mentioned above.

The occurrence of false negative results with HCF may be due to circulating immunocomplexes [39,40,41] or circulating antigens [42,43]. 50-60% of false negative was reported [44,45]. Another hypothesis for the lack of seroreactivity of some hydatid patients could be related in part to the slow growth rate of cysts [46,47,48].

Our study confirmed that IB is a serological technique with a high level of sensitivity (90%) since it was negative only with two out of sera from hydatid patients. Our results showed also that Ag 5 elicits high percentage of positivity as it has been reported by others [14]. In contrast, a low reactivity of antigen B (55%) was obtained in comparison to that described by other studies whose have used IB or immunoprecipitation [48,49]. However, the sensitivity (40%) reported in our study was within the range of the results (33.7%) described by others [20]. The Ag B seem to be important in the humoral response to *E. granulosus* [22,50,51].

Specificity evaluation of total IgG antibodies against eggs, protoscoleces and HCF, by IB, resulted in one positive reaction with sera from subject with viral hepatitis. This result correlated with the high absorbance value obtained in ELISA with this serum. The profile of the three antigens used was identical to that described with hydatid

patients. This reactivity is likely specific and this patient has probably an asymptomatic hydatid cyst [52,53,54].

## 5. Conclusion

Qualitative analysis of IgG response in hydatid patients using IB revealed remarkably different profiles between eggs, protoscoleces and hydatid fluid. The antigens 40, 70 and 100 kDa seem to be specific to eggs. This may be interesting to investigate early versus chronic (long-term) hydatid infection. A more rigorous investigation is required to test their putative specificity. Experiments are in progress to identify those EA recognized during the early steps of infection by experimentally infected lambs. The current study indicated also that ELISA test using eggs, has an excellent specificity. However, its poor sensitivity limits its diagnostic value in CE.

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## Abbreviations

Cystic echinococcosis: CE, Hydatid cyst fluid: HCF, Protoscoleces antigens: PA, Egg antigens: EA, Immunoblot: IB.

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