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ORIGINAL ARTICLE

Animal hydatid cyst genotypes as a potential substitute for human hydatid cyst as a source of antigen for diagnosis of zoonotichydatidosis

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Abstract The Diagnosis of hydatidosis is still an unsolved issue due to difficulties in obtaining of patient's hydatid cyst appropriate for antigen extraction. This study evaluated the suitability of HC protoscolices somatic antigens (HCPsS-Ag) fractions from animal origin to substitute that extracted from HC of patients in diagnosis of hydatidosis using enzyme-linked immunoelectro-transfer blot and Enzyme-linked immunosorbent assay (ELISA). Eight fractions in HC-G6 from patients react specifically versus HC-G6 infected patient's sera. Five of them (28, 32, 38, 59 and 89 Kilo Dalton (KDa) and two of them (28 KDa and 45 KDa) reacted versus HC-G1 and HC-G4 infected sheep and equine sera, respectively. Six fractions in HCPsS-Ag-G1 of sheep react versus HC-G1 sheep infected sera, four (28, 32, 52 and 58 KDa) and two of them reacted versus HC-G6 and HC-G4 infected patient and equine sera, respectively. Two fractions only in HCPsS-Ag-G4 of equine reacted versus infected human and sheep sera. This fraction displayed the same degree of ELISA value versus different infected sera with a significantly perfect classification for *kappa* agreement and non-statistically significant difference ($p \geq 0.05$) for ELISA Optical density values of the positive samples without cross-reaction versus other parasites antibodies in sera. HCPsS-Ag from HC genotypes that developed in humans and animals as HC-G6 and HC-G1 can substitute each other for diagnosis of infection than antigens extracted from non-zoonotic HC-G4. The fraction at 28 KDa is the only fraction that can be extracted from any animals HC and used in diagnosis of zoonotic hydatidosis.

Keywords Antigen fraction EITB · ELISA · Hydatidosis · Human and animal protoscolices

Introduction

Hydatidosis or cystic echinococcosis is a silent cyclo-zoonotic disease-causing public health problem among humans and animals in underdeveloped countries all over the world. The disease is caused by the larvae HC of *Echinococcus* species. This tapeworm developed in the small intestine of dogs as the final host and is transmitted in a synanthropic cycle between dogs, humans, and livestock (Siracusano et al. 2012).

Cystic echinococcosis infection is mostly asymptomatic, with variable and non-specific symptoms. Recently, imaging methods associated with immunological methods are described as a tool for confirming clinical symptoms for the diagnosis in patients (Fotoohi et al. 2013). Due to difficulty in obtaining fresh HC from infected patients suitable for antigen extractions, several serological tests have described the suitability of antigens extracted from HC of animal origin to substitute that from human among serological diagnosis of infection in patients (Karami et al. 2019). Sheep HC has been used routinely to prepare and standardize antigen. Moreover, HC of bovine or camel origin was used successfully as a source of antigens for diagnosing the specific anti-HC-IgG-antibodies (anti-HC-IgG-Abs) in patients using ELISA with variable degrees of sensitivity and specificity without relation to the effect of

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HC genotypes on the degree of diagnostic accuracy of these antigens (Bauomi et al. 2015),

Based on molecular phylogeny, ten *Echinococcus granulosus (E.granulosus)* genotypes (G1 to G10) have been reported to infect different intermediate hosts (IH) species. They were recognized in four main species: *E. granulosus* sensu stricto (G1, G2, and G3), *E.equinus* (G4), *E.ortleppi* (G5) and *E.canadensis* (G6 to G10) (Carmena and Cardona 2014). In Egypt Amer et al. (2015) demonstrated that *E. canadensis* (G6 genotype) was the common species infected camel and 40% of sheep while 60% of sheep cysts were *E. granulosus* sensu stricto (G1 genotype). Moreover, *E. canadensis* (G6 genotype) was detected in 96.8% of the examined human isolate. While all equine *Echinococcus* were identified as (G4) (Aaty et al. 2012). This genotype did not describe infecting humans (Manterola and Otzen 2016).

Anti-HC-IgG-Abs in HC infected patients were reacting specifically versus variable protein fractions present in fractionated HC- protoscolices Somatic antigen (HCPsS-Ag) extracted from the animal using EITB. The fraction of MW 31 KDa in sheep, 45 KDa in equine, and 125 KDa in camel and horse not in sheep HCPsS-Ag were reacted specifically versus HC infected patient sera (Rafiei and Craig 2002). The fraction at 27.5 KDa MW in HCPsS-Ag of sheep origin appears more specific and sensitive for capturing of anti-HC IgG-Abs in sera of infected sheep (80% and 75%) than in the serum of infected human (66.7% and 62.5%) using ELISA (Bauomi et al. 2015). Most of these authors did not investigate the relationship between these specific fractions and the genotypes of cyst used for the extraction of these antigens.

ELISA is one of the most common, easily applicable field tests. Its accuracy depends on several factors including the stability, and specificity of the used antigen especially if it is compared with other techniques such as EITB (Sangaran et al. 2017) or DNA extraction technique (Amer et al. 2015). EITB technique has high diagnostic sensitivity but it is non-applicable in field settings as its time consuming and can't be applied for many samples. Sangaran et al. (2017) demonstrated a special improvement to the antigen used in ELISA increasing its sensitivity by separation of specific protein fraction after identification by EITB and using it in the coating of the ELISA plate after elution and concentration. This modification improves the ELISA to be used as an accurate test, gathering the benefits of both ELISA and EITB, and can be applied for many samples.

The present study was carried out to evaluate the suitability of HCPsS-Ag derived from identified animal HC genotype to substitute that extracted from HC of patients in diagnosis of zoonotic hydatidosis. The levels of specific antigenic similarity in fractionated HCPsS-Ag derived from HC-G1 of sheep and HC-G4 from equines were compared with that present in similar antigen extracted from HC-G6 obtained from infected patients using EITB. The specific common fractions between different genotypes were eluted and concentrated and evaluated in diagnosis of infection using ELISA.

Materials and methods

Collection of HC and extraction of HCPs

Fresh surgical removed HC from the lung and liver of previously sonographic diagnosed patients in the governor and private hospital were collected. Moreover, HC extracted from the liver of infected equine freshly slaughtered in the Giza zoo abattoir and from lung and liver of sheep slaughtered in Cairo abattoir was collected also. Protoscolices (Ps) were extracted from each fertile cyst separately via aseptic cyst puncture in Dept. of Parasitology, Faculty of Vet. Med., After microscopic examination of the materials, active Ps with clear HC fluids were selected for the study. Representing sample from each separate Ps in Phosphate buffered saline (PBS) (pH 7.2), were sent to the Department of Biotechnology, College of Veterinary Medicine Cairo university for genotyping identification.

HC genotypes selected for the study

The obtained protoscolices (from human, sheep and equine) were genotyped versus reference samples in the Department of Biotechnology, College of Veterinary Medicine Cairo University by Multiplex PCR (mPCR) according to the protocol of Boubaker et al. (2013). DNAs were extracted from the collected cysts directly after necropsy using QIAamp DNA mini kit (Qiagen, Hilden, Germany) and therefore the procedure was done in line with the directions of the manufacturer. The extracted DNAs were entered mPCR for identification of *E. granulosus* sensu stricto (G1), *E. equinus* (G4), *E. canadensis* (G6) using the subsequent sets of primers: For*E. granulosus* sensu stricto (G1): 5′ TCCTAACATGCCTTGGTAT 3′; 5′ GTTACAGCCTTGATCACG 3′, For *E. equinus* (G4): 5′ GTTGGGTTGGATGTT 3′; 5′ CAAAACAGGATCACTCTT 3′ and for (G6 strain): 5′ GGCCTTCATCTCCATAATA 3′ and 5′ ATGAAGAGTTTGAAACTAAAG 3′. The reaction was administrated using EmeraldAmp GT PCR master mix (Takara, Japan) in an exceedingly total volume 25 µl with a reaction mixture contained 5 µl of sample DNA as a template and 10 pmol of every primer (Metabion, Germany). The amplification conditions were included the subsequent

thermal profile: 94 °C for 3 min, 25 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s and a final extension step for five min at 72 °C. After the amplification step, the amplicons were entered electrophoresis step in 1.5% agarose and specific bands were noted at 706, 124 bp and 617 bp for G1, G4 and G6 strain, respectively.

HCPsS-Ag preparation

According to Hassanain et al. (2016), HCPs were separated from HC-fluid by centrifugation at 1500*g* for 30 min at 4 °C. The sedimented Ps were washed with PBS 7.2 then exposed to 3 cycles of freezing and thawing, then sonicated in an ice bath by using "Cole Parmer ultrasonic Homogenizer (under 150-W interrupted pulse output in 10-s bursts with five seconds intervals). After centrifugation at 14,500*g* for 30 min., the supernatant was collected as soluble HCPs somatic Ag (HCPsS-Ag). It was dialyzed versus5mM Tris–HCl (pH 7.4) for 48 h. at 4 °C. Its protein contents were measured using the Bradford method (Bradford 1976). Then dispensed in one ml plastic vials and stored at − 20 °C until use.

Fractionation of antigens and transferring of proteins

The prepared PsS-Ag from human, sheep, and equine HC were resolved separately using 1.0 mm thickness, SDS-PAGE according to Laemmli (1970). The comb was adjusted as one small well for the MW standard and one large for the sample. The fractionated proteins were transferred onto the nitrocellulose sheet (NC) at 10 V, 100 mA overnight at 4 °C, according to Towbin et al. (1979). The NC sheet was dried then stored in freezing until use.

Determination of specific protein fractions using EITB

Longitudinal NC strips (10 × 0.4 cm) containing the fractionated HCPsS-Ag was cut out. Four types of sera were reacted versus each fractionated Ag as a known positive serum sample from humans, sheep, and equine as well as negative control one. The strips were incubated versus each serum samples at 1:100 dilutions and Horseradish peroxidase-conjugated antihuman or anti-sheep or anti equine IgG (Sigma, USA) was used as conjugate at 1:1000 dilutions in blocking buffer. Fractions reacting positively versus positive sera and did not react versus negative one is considered as specific protein fractions. Percentage of polypeptides in specific fractions that reacted versus its natural infected sera after EITB were estimated using Linear Chromoscan (Joyce-Loebl – chromoscan-3 S/N 3225 REU 6.1 USA).

Separation of specific protein bands from the gel after fractionation

SDS-PAGE analysis of the three crude HCPsS-Ag under reduced conditions was done as before once the gel ran its full length, the level of the required protein fraction was determined after staining of this gel strip by Commassi blue stain according to Tsai and Frasch (1982). A transverse gel strip containing the proteins corresponding to MW of 28 KDa was determined then cut out horizontally across the whole gel. Protein contents in this gel strip were eluted by transferring this gel strip to the elution tube membrane of 6–8 MW cut off (Spectrum Medical Inc., Los Angeles, CA 900060 USA) filled with PBS (pH 7.4) using Bio-Rad elution unit at 10 V, 100 mA overnight at 4 °C. The eluted Ag was concentrated using poly-ethylene–glycol in the same previous tubing according to Hassanain et al. (2016). The protein content of the eluted concentrated material (28 KDa antigen (EC- 28 KDa Ag) was used in the coating of the ELISA plate to be used in a reexamination of the previously tested serum samples.

Indirect ELISA

EC 28 KDa-Ag were evaluated in capturing of anti-HC-IgG –Abs in infected sera using indirect ELISA according to the protocol of El-Kattan et al. (2020) with little modifications. The condition of the test was optimized after checkerboard titration at a volume of 100 µl/well. Horseradish peroxidase HRP-conjugated anti-sheep, or anti-equine or anti-human IgG (Sigma, USA) was used at 1:5000 dilutions. The reactions were determined by the addition of 100 µl/well of the substrate (O-phenylene diamine dihydrochloride (OPD), Sigma, USA) and hydrogen peroxide (H_2O_2). The Optical density (OD) values were measured at 450 nm with a full automated Titerteckmultiskan ELISA microplate reader. Serum samples were tested in duplicate. The cut-off value was determined individually for each antigen versus its infected host serum. Cut-off formula = 2 × MEAN of negative controls (Lardeux et al. 2016) Depending on the cutoff values, statistical analysis was carried out as 0.1396, 0.4052 and 0.4352 (OD values) for positive sera versus EC-28 KDa-Ag for G6, G1 and G4 genotypes respectively.

Tested sera

All serum samples included in the test were previously confirmed as they are free from any other parasitic infections.

Human serum samples

Serum was separated from blood samples collected from attendants of tropical medicine outpatient clinic, Cairo University Hospitals, and other samples collected by personal communications from private medical diagnostic laboratories as the following: Twenty serum samples were selected from 31 surgically confirmed liver and lung HC infected patients. Sera of patients infected by active non-calcified cyst HC which previously confirmed as genotype G6 only were selected for the study, 40 samples from suspected patients have positive Casoni test with a history of contact with dogs and positive clinical-pathological data for leukocytosis, eosinophilia and increased levels of muscle enzymes but all of them are negative sonography for the presence of HC. Another 20 samples from patients having 1–3 *Schistosoma mansoni* (*S. mansoni*) eggs in their stool and ten samples from the patient's PCR positive for Hepatitis C virus infection. Moreover, 20 samples from healthy donors were used as a negative control.

Selected animal serum samples

During several visits to Cairo abattoir and Giza zoo slaughterhouses, identified blood and fecal samples were collected from the slaughtered sheep and equine. After postmortem inspection of their carcasses, blood samples of animals contained HC only in their lungs or livers and those have *Cysticercus* or *Fasciola* only were separated. After genotyping to the obtained HC, sera of 20 sheep infected only by active non-calcified HC in their livers or lungs were selected for the study. Moreover, 10 samples from those infected by *Cysticercus ovis* only, and 10 sera of an animal infected by *F. gigantica* only as well as 10 sera from healthy non-infected sheep samples were selected for the study also. In the same way, 20 serum samples were collected from equine infected by active non-calcified HC in their liver only (identified as HC-G4), five serum samples from equine infected by *Sarcocystis* muscle cyst only and 10 samples from healthy non-infected ones were selected for the study.

Fecal and stool examination

Fecal and stool samples of the cases enrolled in the study were examined parasitologically for diagnosis of any other infection using the Fluke finder technique (Welch et al. 1987) for diagnosis of large size eggs and the concentration flotation technique (Soulsby 1982) was adopted for the diagnosis of the other parasitic stages.

Statistical analysis

Statistical analyses of screening were performed using Chi-square (χ^2) and Kappa agreement tests. The degree of Kappa agreement was measured according to the Landis and Koch scale (Landis and Koch 1977). Statistical comparison between the median of the different screening tests was made by independent sample Kruskal–Wallis test and significance values have been adjusted by the Bonferroni correction for multiple tests. Values of $p \leq 0.05$ were considered statistically significant. All statistical analyses were performed on SPSS version 26 software (SPSS Inc, Chicago, IL, USA).

Results

The data in Fig. 1a revealed that the treatment of the fractionated HCPsS-Ag extracted from the HC-G6 genotype of patients versus sera of HC-G6 infected patients using EITB revealed 8 specific bands corresponding to MW from 23 to 89 KDa, did not react versus negative human sera. Among these fractions, five fractions at MW of 28, 32, 38, 59 and 89 KDa were reacted also versus sera of sheep infected by the HC-G1 genotype. At the same time, two fractions only at MW of 28 and 45 KDa were reacted versus HC-G4 infected equine serum samples.

By the same way, the fractionated HCPsS-Ag of sheep HC-G1 genotype was reacted versus infected sheep serum samples (Fig. 1b) revealed seven fractions corresponding to the MW at 28, 32, 42, 46, 52, 68 and 85 KDa. These fractions except those of MW of 68 KDa considered being specific since they did not recognize by negative sheep sera. Four fractions from them (28, 32, 34–42, and 52 KDa) were specifically reacted also versus HC-G6 infected patient sera. The fractions at MW of 28 and 85 KDa only from them were reacted versus HC-G4 infected equine serum samples. Moreover, the fraction at MW of 85 KDa is a common band reacted specifically versus infected sheep and equine sera, the fraction of MW 58 KDa in this sheep Ag did not react versus infected sheep sera but on the contrary, it reacts specifically versus infected equine and human sera.

Reacting of fractionated HCPsS-Ag extracted from HC-G4 of equine versus infected equine sera revealed 5 specific fractions at MW of 28 KDa to 100 KDa. From these fractions, that of 48 and 45 KDa was reacting specifically versus HC infected sheep and human serum respectively. While another specific fraction at MW of 32 KDa in this equine Ag did not react versus infected equine sera but it reacts specifically versus infected sheep and

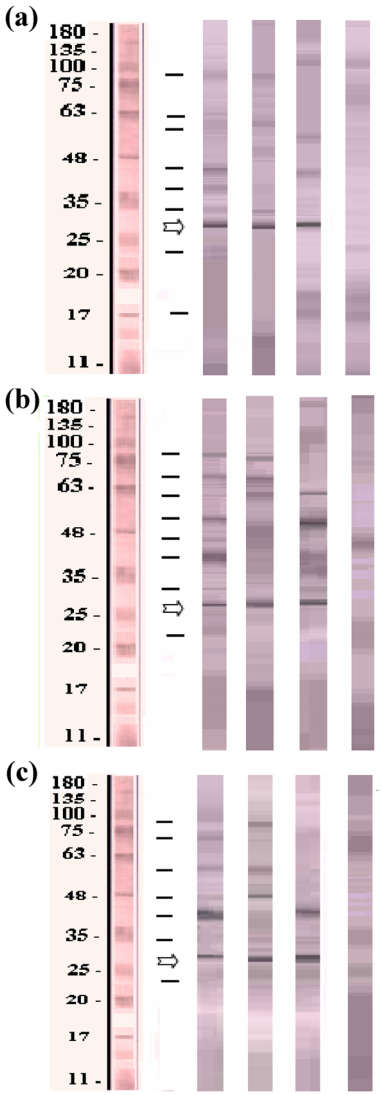

Fig. 1 Reacted polypeptides in fractionated HCPsS-Ag of different genotypes versus human and animal sera using EITB. **a** Reacted polypeptides in fractionated (G6) HCPsS-Ag of human on NC strips versus HC infected patient sera (Lane 1), HC infected sheep sera. (Lane 2), HC infected equine sera (Lane 3) and sera of healthy people (Lane 4). MwSt. = Low Molecular weight protein slandered (Sigma). **b** Reacted polypeptides in fractionated (G1) HCPsS-Ag of sheep on NC strips treated versus HC infected sheep sera. (Lane 1), HC infected equine sera. (Lane 2), HC infected patient sera. (Lane 3) and negative sheep sera (Lane 4). MwSt. = Low Molecular weight protein slandered (Sigma). **c** Reacted polypeptides in fractionated (G4) HCPsS-Ag of equine on NC strips versus HC infected horse sera (Lane 1), HC infected sheep sera. (Lane 2), HC infected patient sera (Lane 3) and non-infected equine sera (Lane 4). MwSt. = Low Molecular weight protein slandered (Sigma)

human serum samples (Fig. 1c). It was important to demonstrate that the HC-G4 genotype of equine showing the lowest number of fractions that can react specifically versus HC-G6 and HC-G1 infected human and sheep sera. Moreover, the fraction of 28 KDa is the only common specific fraction present in HCPsS-Ag of different origin and react specifically versus anti-HC-IgG-Abs present in HC infected human and animal serum samples using EITB.

Estimation to the percentage of the reacted specific polypeptides in this fraction using Linear Chromoscan (Joyce-Loebl – chromoscan-3 S/N 3225 REU 6.1 USA) in strips treated versus sera from the same host origin as described in Table 1 and Fig. 2 revealed another advantage for this fraction as it appears to contain a nearly constant amount of specific polypeptides as it ranged from 17.12 to 18.11%.

Evaluating the diagnostic efficacy of EC-28 KDa-Ag using ELISA

Testing the efficacy of this Ag in the diagnosis of anti-HC-IgG-Abs in the previous serum samples using ELISA (Table 2) revealed that, while this fraction originated from three different HC genotypes, it has the same value in capturing anti-HC-IgG-Abs in different screened serum samples without any effect to its host origin. Concerning the agreement analysis between these antigens, there are no statistically significant differences in the calculated *kappa* values between testing of different sera versus EC-28 KDa-Ag of different genotypes ($K = 1.000$, $p = 0.005$ to 0.001) (Table 5). The diagnostic efficacy of the three antigens is similar even when calculated statistically using the results obtained versus HCPsS-Ag of human origin as the gold standard for evaluation. At the same time, the data revealed marked similarity in the *kappa* values with significant perfect *kappa* agreement ($K = 1.000$, $p = 0.001$) at using the antigen of human or animal origin in diagnoses of infection in suspected patients (Table 3).

Statistical analysis of the Median ELISA OD values obtained using EC-28KDa-Ag of different genotype in the diagnosis of infection

Statistical analysis was done depending on the cutoff values as 0.1396, 0.4052 and 0.4352 for positive sera versus EC-28 KDa-Ag of G6, G1 and G4 genotypes respectively. Statistical analysis of the obtained Median ELISA OD values of the positive samples showing high but non-significant ($p \leq 0.05$) difference in ELISA absorbance value (OD) recorded during testing of each Ag versus infected serum samples obtained from the same host origin. Inspection of the same serum samples versus the three antigens revealed a non-statistically significant difference ($p \leq 0.05$) between the obtained media ELISA OD values. Screening of infected patient serum versus EC-28 KDa-Ag of G6, G1 and G4 genotypes revealed nearly the same median ELISA OD value with a slight non-significant

Table 1 Densitometric concentration of the amount of specific polypeptide present in the band of 28 KDa. Diagnosed in fractionated HCPsS-Ag of different origin (Aperature width = 3.0 mm) (Chromoscan 3)

Peak data	NC strip containing Fractionated HCPsS-Ag of		
	(G1) from sheep versus sheep infected sera	(G6) from human versus patient sera	(G4) from equine versus infected horse sera
Peak no./total peak no	9/11	6/9	8/10
Position	6.4 mm	6.5 mm	6.5 mm
Hight	11.1 mm	10.1 mm	9.2 mm
REL%	18.11%	17.42%	17.12%

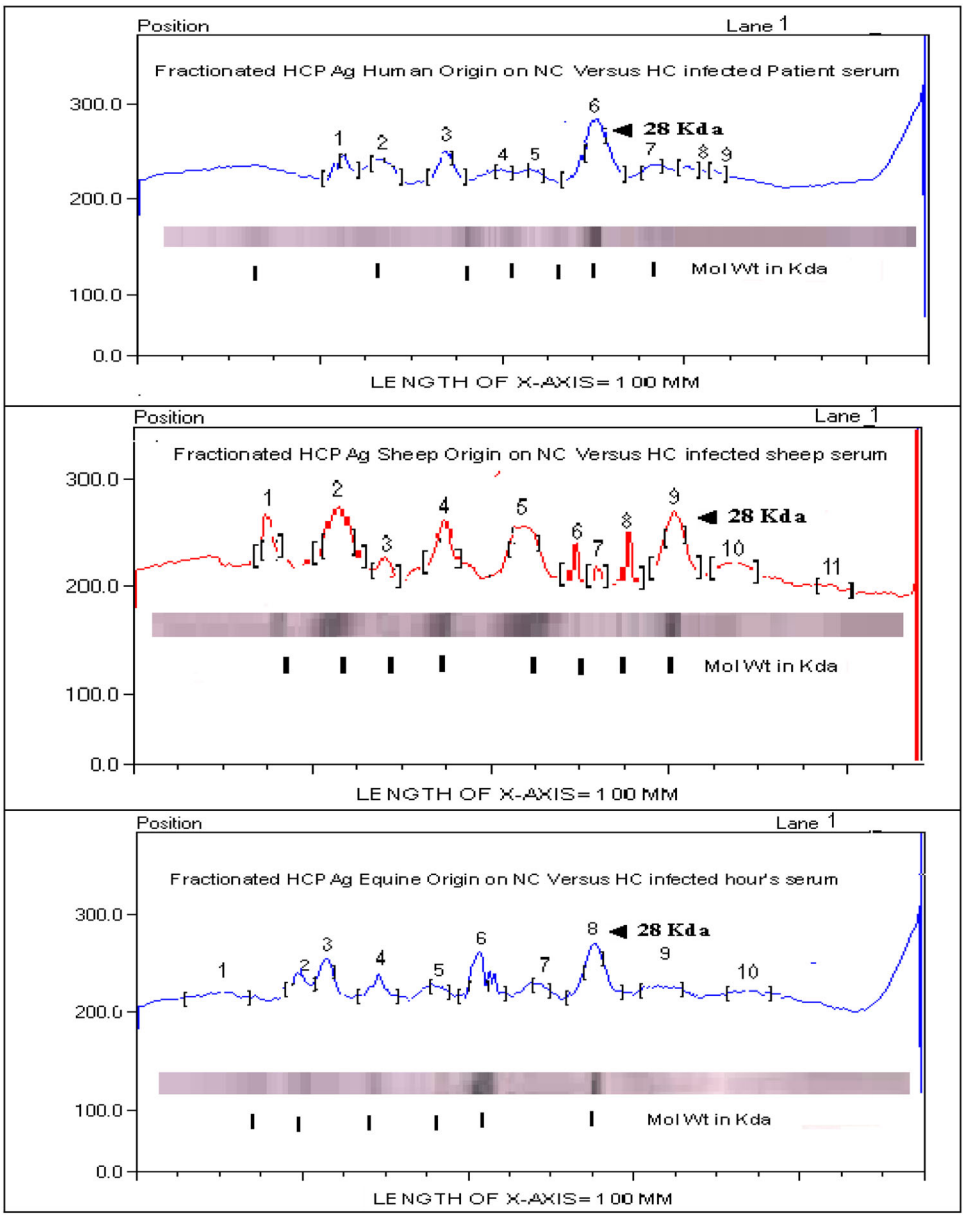

Fig. 2 Densitometric concentration percentage of the amount of specific polypeptide present in the band of 28 KDa diagnosed in fractionated HCPsS-Ag of different origin versus infected sera of the same host (Aperature width = 3.0 mm) (Linear Chromoscan 3)

Table 2 Efficacy of EC-28 KDa-Ag from different HC genotypes in the detection of anti-HC-IgG-Abs in selected human and animal sera using ELISA

Tested serum samples		The reaction of EC-28 KDa-Ag originated from HC of		
		Human (G6)	Sheep (G1)	Equine (G4)
HC infected human (n = 20)	Positive	19 (95%)	19 (95%)	19 (95%)
	Negative	1 (5%)	1 (5%)	1 (5%)
HC infected sheep (n = 20)	Positive	19 (95%)	19 (95%)	19 (95%)
	Negative	1 (5%)	1 (5%)	1 (5%)
HC infected equine (n = 20)	Positive	19 (95%)	19 (95%)	19 (95%)
	Negative	1 (5%)	1 (5%)	1 (5%)
Suspected HC human patients (n = 40)	Positive	28 (70%)	28(70%)	28(70%)
	Negative	12 (30%)	12(30%)	12(30%)

Table 3 Statistical analysis for the reaction of EC-28 KDa-Ag from the different HC genotypes in diagnosis of anti-HC-IgG-Abs in selected human and animal sera

Samples	Gold standard (EC-28 KDa-Ag)	Reaction Versus EC-28 KDa Ag originated from	Pearson Chi-square	*Kappa* value	*p* value
HC infected human (n = 20)	(Human)	Sheep (G1)	20.000	1.000	0.001
		Equine (G4)	20.000	1.000	0.001
HC infected sheep (n = 20)	(Sheep)	Human (G6)	20.000	1.000	0.001
		Equine (G4)	20.000	1.000	0.001
HC infected equine (n = 20)	(equine)	Human (G6)	20.000	1.000	0.001
		Sheep (G1)	20.000	1.000	0.001
Suspected HC patients (n = 40)	(Human)	Sheep (G1)	40.000	1.000	0.001
		Equine (G4)	40.000	1.000	0.001

Table 4 Median ELISA OD values using EC-28 KDa-Ag from the different HC genotypes in diagnosis of anti-HC-IgG-Abs in selected human and animal sera

Samples	EC-28 KDa-Ag extracted from		
	(Human) (G6)	(Sheep) (G1)	(Equine) (G4)
HC infected human (n = 20)	$0.590^{Aa} \pm 0.09$	$0.5835^{Aab} \pm 0.02$	$0.5065^{Aab} \pm 0.13$
HC infected sheep (n = 20)	$0.5015^{Aa} \pm 0.03$	$0.6980^{Ba} \pm 0.02$	$0.6030^{Ca} \pm 0.03$
HC infected equine (n = 20)	$0.4995^{Aa} \pm 0.03$	$0.6960^{Ba} \pm 0.04$	$0.8075^{Cc} \pm 0.04$
Suspected HC human patients (n = 40)	$0.5905^{Aa} \pm 0.60$	$0.4965^{Ab} \pm 0.31$	$0.4965^{Ab} \pm 0.31$

Data represented as median ± IQR (interquartile range), A column with different small letters are significantly different, Row with different capital letters are significantly different at p value ≤ 0.05(independent sample Kruskal–Wallis test, Significance values have been adjusted by the Bonferroni correction for multiple tests

increase versus that of the human than that of animal origin. By the same way using EC-28 KDa-Ag extracted from G1 or G4 genotype gives higher median ELISA OD values versus anti-HC-IgG-Abs in sheep or equine sera than that obtained versus human sera with a slightly significant difference ($p \leq 0.05$). At the same time, there was no statistically significant difference ($p \leq 0.05$) was recorded in these values at using EC-28 KDa-Ag extracted from G1 or G4 genotypes in the diagnosis of infection in infected or suspected human sera (Table 4).

Table 5 Level of cross-reaction between EC-28 KDa-Ag of different genotypes and specific anti-bodies of other parasites present in investigated serum samples

Tested sera non-infected by HC but infected by	No. of + Ve and % of specificity versus EC-28 KDa-Ag of Genotype		
	(G1) Sheep	(G4) equine	(G6) Human
Cysticercus ovis infected sheep (n = 10)	(1) 90%	(1) 90%	(1) 90%
F. gigantica infected sheep (n = 10)	(1) 90%	(1) 90%	(1) 90%
Sarcocystis infected equine (n = 5)	(1) 80%	(1) 80%	(0) 100%
S. mansoni infected patients (n = 20)	(0) 100%		
Hepatitis C infected (n = 10)	(0) 100%		
- Ve sheep(n = 10)	(0) 100%		
- Ve equine (n = 10)	(0) 100%		
Healthy donors (n = 20)	(0) 100%		
Mean specificity (n = 95)	(86) 90.53%		

Cross reaction between EC-28 KDa-Ag versus antibodies of other parasites

Evaluating the ability of the tested fraction (EC-28 KDa-Ag) that extracted from the three HC-genotypes in cross-reaction with other anti-parasite Abs which may be present in tested serum samples, Table 5 revealed that no cross-reaction between this antigen and antibodies present in the patient sera infected by *S. mansoni* or HCV, but false-positive results (one case) were diagnosed as HC infected from sheep sera of animals infected by *C. ovis* or *F. gigantica*. Also, with one equine serum from animals infected by *Sarcocystis* muscle cyst. While no false-positive results were recorded on screening of control negative serum samples of different sources. Collectively the mean diagnostic specificity of this fraction on the level of the tested sera reached 90.53% (Table 5).

Discussion

The diagnosis of hydatidosis stills an unsolved problem despite the development of many serological techniques. One of the serological technique problems in the diagnosis of zoonotic hydatidosis is obtaining HC from patients in enough numbers and a condition suitable for antigen extraction and preparation. For this reason, most of the available serological techniques were depend on using of HC antigens extracted from animals to diagnose the infection in patients (Fotoohi et al. 2013 and Bauomi et al. 2015). HC present in 10 intraspecific genotypes of *E. granulosus* (G1 to G10), with variable important phenotypic variation in characteristics of the biological cycle, transmission dynamics, intermediate host specificity, pathogenicity, antigenicity, and in the measures required for control of the disease (Manterola and Otzen 2016). These variations must be reflected in the antigens extracted from animal HC to use for the diagnosis of human hydatidosis. The selection of protoscolices antigen rather than HC-fluid for evaluation was related to what mentioned by Zhang et al. (2012) as HC fluid Ag extracted from HC of human origin considered unsuitable for diagnosis because it contained host proteins such as IgG which may be cross-reacted with the specific immunoglobulin present in sera of HC infected patients. Aiming to determine the value of HC extracted from animals to substitute that extracted from human as a source of diagnostic Ag, in the first part of the study, the level of antigenic similarity between three different HC genotypes were investigated versus infected human and animal sera using EITB technique.

The three selected HC genotypes are HC-G6 as it is the most common genotype infecting human in Egypt, HC-G1 of sheep as it commonly distributed genotype in animal and also recorded to infect human (Amer et al. 2015), the third one is HC-G4 of equine as it non-zoonotic genotype as recorded by Manterola and Otzen (2016) not infect human beings. Genotyping of these used antigens will be published in other related work.

Eight specific fractions at MW of 23–89 KDa in HCPsS-Ag extracted from HC-G6 of patients that react specifically versus anti-HC-IgG-Abs present in HC-G6 infected patient sera, five fractions out from them at MW of 28–89 KDa were react also versus sera of sheep infected by HC-G1. While two fractions only (28 and 45 KDa) from them were reacted versus HC-G4 infected equine sera. At the same time, from seven fractions at MW of 28–85 KDa in fractionated HCPsS-Ag extracted from HC-G1 of sheep

reacted specifically versus infected sheep sera, five out of them at MW of 28–52 KDa were reacted specifically versus HC-G6 infected patients. While two fractions (28 and 85 KDa) were reacted versus HC-G4 infected equine sera. Finally, from five specific fractions in fractionated HC-G4 of equine that react specifically versus infected HC-G4 infected equine sera, two fractions only (28 and 45 KDa) and (28 and 48 KDa) were reacted specifically versus HC-G6 infected human and HC-G1 infected sheep sera respectively. Most of these specific fractions were in agreement with that previously mentioned by Carmena et al. (2004), Rafiei and Craig (2002), and Bauomi et al. (2015). Moreover, it was interested in recommended that the fraction of 58 KDa in G1 of sheep reacted specifically versus infected equine and human not versus sheep sera and the fraction of 32 KDa in G4 reacted versus G1 and G6 not for G4 infected sera. These data were in agreement with Bauomi et al. (2015) and Houssaini (2015).

Increase the number of common sharing specific fractions recorded in this part of the study concerning reacting of fractionated HC-G6 of patients versus HC-G1 infected sheep sera and those of fractionated HC-G1 of sheep versus HC-G6 infected patients demonstrated the suitability of sheep HC-G1 to replace that extracted from HC-G6 of patients in the diagnosis of zoonotic hydatidosis. This level of common fractions between these two genotypes (G6 and G1) was not recorded at testing the same sera versus HCPsS-Ag extracted from HC-G4 of equine. Using of HC antigens from sheep to diagnose infection in human agreed with Carmena et al. (2004), Carmena et al. (2005) and Rojas et al. (2014) as they recorded a high level of shared antigenic components in HC protoscolices (sheep origin) somatic antigens react specifically versus anti-HC- Abs in sera of HC infected patients.

In the authors' opinion, the compatibility between HC-G6 and G1 genotypes may be related to the ability of both genotypes to develop in humans and sheep. This was previously mentioned by Aaty et al. (2012) and Rojas et al. (2014) the condition which did not record versus HC-G4 antigenic fractions as the equine genotype did not record to develop in human as mentioned by Manterola and Otzen (2016).

Among the entire previous common specific fraction, the fraction at MW of 28 KDa in the three different HC genotypes (including HC-G4) reacted specifically versus anti-HC-IgG-Abs in sera of humans, sheep and equine infected by the 3 different genotypes. Moreover, the amount of specific reacted polypeptides in these fractions appears nearly constant versus different sera after measuring its polypeptide density in them using the Linear Chromos can method. This fraction present at the level of a specific MW region (27.5–29 KDa) as that previously determined by Bauomi et al. (2015). The little variation in the MW between the previous study and this study may be related to differences in the tested genotype of *Echinococcus* species included in the study of Bauomi et al. (2015).

In a time where no statistically significant difference in the used EC-28 KDa-Ag of different origin in the diagnosis of infection in infected human and animal sera, statistical analysis of the obtained Median ELISA OD values of the positive samples showing a high but non-significant ($p \leq 0.05$) difference in ELISA OD was recorded during testing of each Ag versus infected serum samples obtained from the same host origin. In the authors' opinion and agreement with Fotoohi et al. (2013) and Carmena et al. (2005), this variation may be related to the level of specific antibody titer in these serum samples. Further study will be done to examine the diagnostic accuracy of this test in a large cohort study.

Investigating the ability of this fraction to exclude the cross-reacted anti-bodies that originate from some common parasites infect human and animals and follow closely related HC migratory way and causing some level of hepatic disturbances such as *Schistosoma*, Viral hepatitis and *Cycticerci* revealed that this fraction showed no cross-reaction versus anti- *S. mansoni* or anti- HCV antibodies present in these patient sera. A low level of cross-reaction was recorded in sheep sera of animals infected by *C. ovis* or *F. gigantica* and one equine serum from animals infected by *Sarcocystis* muscle cyst. The recorded low level of cross-reaction with the Ab of other parasites especially versus infected animal sera may be related to the presence of in-apparent infection by HC in these animals.

Conclusion

HCPsS-Ag extracted from HC genotypes that are recorded to develop in each of humans and animals as HC-G6 and HC-G1, can substitute each other as a source of antigens for diagnosis of infection in patients than similar antigens that extracted from non-zoonotic genotypes as HC-G4 of equine. The fraction of 28 KDa is the only common non-host dependent antigen that can be extracted from easily available HC of animal's origin to use in the diagnosis of zoonotic hydatidosis. This fraction contains the same concentration of specific polypeptides and it can produce as recombinant or chemically sensitized for commercial diagnostic kits production.

Author contributions Mohamed M. El-Bahy was the leader of the research team, he designed the study, supervise the work, help in preparing and revising the Manuscript. Reem, M. Ramadan; Marwa, M. Khalifa and Dina, M. El-Akkad carried out Collection of Hydatid cyst and extraction of Hydatid cyst protoscolices, antigen extraction,

fractionation of antigens and transferring of proteins, determination of specific protein fractions, Enzyme-linked immunosorbent assay and DNA extraction. Azza, M. Abdel-Wahab carried out the Polymerase chain reaction work. All authors revised and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical standards The protocol of this study was approved by the Faculty of Medicine Cairo University Ethics Committee after taking consent from local health authorities and all participants. Moreover, the used procedures of handling the animals, collection of samples, and the use of the patients' samples in this study were assessed and approved by the institutional review board of the Institutional Animal Care and Use Ethical Committee (Vet CU20022020132) of Cairo University. The present study was conducted during the period from December 2019 to March 2020 in the Department of Parasitology, Faculty of Veterinary Medicine, Cairo University.

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