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Detection of *Echinococcus granulosus* sensu *lato* infection by using extracts derived from a protoscoleces G1 cell line

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Abstract

Cystic echinococcosis (CE) can be diagnosed by means of several serological approaches, but their results vary among laboratories due to the molecular characteristics of the reference antigens used. Thus, this study aimed to address both the relevance of an EGPE cell line previously obtained from *Echinococcus granulosus* protoscoleces G1 and the complexity of the immune response by using two different in vitro growth stages as separate sources of parasite antigens. The serum reactivity was investigated by western blotting (WB) in 21 CE patients from an endemic area in a matched case-control design and also in seven experimentally infected sheep and five healthy control sheep. EGPE-antigen-human serum sensitivity by WB was higher than that of hydatid fluid (HF) WB, ELISA and DD5 ($P < .05$, Chi-square test). EGPE protein extract was immunogenic in mice and hyperimmune plasma reacted with HF proteins, and AgB2 expression was detected by molecular analysis. Proteins of 37 to 60 kDa were recognized by 95.24% of the CE patients' sera but, with poor specificity. Statistically significant differences were found between serum protein extract recognition at 7 and 20 days of cell growth. The EGPE cell line is a laboratory source of antigens for improvement of CE serological diagnosis.

KEYWORDS
cell line extract, Cystic echinococcosis, human, immunoblot, parasite, sera, sheep

1 | INTRODUCTION

Cystic echinococcosis (CE), also called hydatidosis, is a zoonotic parasitosis worldwide distributed[1] caused by the larval stage (metacestode) of *Echinococcus granulosus* sensu *lato*. This hermaphrodite worm develops in the intestine of domestic and wild canids, and then its gravid proglottid releases the eggs to the faeces, contaminating the environment. The eggs ingested by the intermediate host hatch in the duodenum, and the oncosphere then penetrates the intestinal wall, migrates, and becomes trapped in the host's organs, where it becomes metacestode. The parasite's cycle is accomplished when the infected organ from the intermediate host is ingested by the definitive host (canids).[2] Humans, as intermediate accidental hosts, who are more exposed to the infection in unsanitary conditions, develop a parasitic disease involving the encystment of the larvae in different organs (the liver in 70%-80% of the cases, the lungs in 20%-30% of the cases and other organs with lower frequency),[3] resulting in a wide spectrum of clinical manifestations. Mitochondrial DNA has so far allowed identifying eight *Echinococcus granulosus* sensu lato genotypes (G1, G3-G8, and G10).[4,5] Four of these genotypes, G1 in sheep, G5 in cattle, G6 in camelids and G7 in swine, have been detected in Argentina.[4]

The metacestode cyst has three membranes: two derived from the parasite tissue, which are laminated and germinal, and the other which is the outer membrane and is originated in the reaction from the host.[6] Protoscoleces, which are embryonic corps, sprout from the germinal layer and isolated calcium-rich cells are released to the hydatid fluid (HF) of the cyst. The composition of the HF may vary and has been correlated with the metacestode stage.[7] The contents of primary cysts can spill by spontaneous rupture, trauma or cyst surgery and secondarily invade other organs of the body.[8,9]

The clinical diagnosis of CE is guided by local epidemiologic data, clinical symptoms, and imaging. Infection is classified by ultrasound according to the expert recommendations for liver cyst localization developed by the WHO, and the image is correlated to the viability of the cyst.[10] However, the disease onset could be undetectable by image methods or may result in misdiagnosis. In addition, the rate of success of the pharmacological treatment of CE needs an early diagnosis of the infection.

Both humans and livestock could be screened for CE by means of serological methods. In livestock, a robust serological method may be beneficial to monitor the disease reinstated in CE-free areas by infected livestock trade, stray dogs, or migrating rural workers carrying infected animals. The current serological methods used for human CE diagnosis have not enough sensitivity, specificity or reproducibility among laboratories due to the use of crude or semi-purified parasite extracts as antigens.[11,12] Alternative methods to improve serological diagnosis of CE have thus been proposed. These include: evaluation of interleukin levels,[13,14] antibody class change,[15,16] antigen identification and purification from freshly extracted metacestodes[17,18] and the use of antigenic recombinant proteins[19,20] or fusion proteins assayed in infected animals[21] in immunodiagnostic assays.

Consistent with the need to improve the serological diagnosis of CE, in our laboratory, we have previously developed a cell line from bovine *E granulosus* G1 protoscoleces, genotyped from its mitochondrial DCO1 sequence, which we called EGPE.[22,23] This cell line grows in axenic liquid medium, developing two defined morphological stages (Figure S1): up to 7 days of growth, cells are organized in clusters of small cells in suspension, a stage referred to as "short," whereas, after 20 days, cells aggregate and attach to the released floating membranes, a stage referred to as "long". Based on this, the aim of this work was to investigate whether the EGPE cell line may be a source of antigens in CE serology, by analysing the reactivity of serum from patients with CE with EGPE protein extracts from these two growth periods in a matched case-control design. Reactivity results were also obtained with EGPE extracts and sera from infected sheep, and the antigenic properties of EGPE extracts were confirmed by antibody reactivity from EGPE immunized mice to HF protein extract. Finally, since some authors consider AgB2 as a hallmark of CE,[24] we attempted to confirm its presence in EGPE cells, to complementarily support the potential of the EGPE extracts as a source of antigenic proteins to improve CE serology.

2 | MATERIALS AND METHODS

2.1 | Human serum samples

Forty-two serum samples were collected from outpatients attending the Municipal Hospital "Ramón Santamarina" in Tandil, a CE endemic area, between 2012 and 2016. Twenty-one of the samples were from CE patients (10 men and 11 women aged 52.57 ± 22.44 years old) whereas the other 21 of the samples were from healthy donors (9 men and 12 women aged 47.86 ± 24.15 years old). The study had a case-control design matched by habitat: urban or rural. Samples were collected after patients and controls gave their informed consent and before patients received CE treatment with albendazole. CE was diagnosed by clinical symptoms and images, and CE diagnosis then confirmed by ultrasound in 90.48% of the patients and 80.95% of the cysts were staged according to WHO.[10] Other complementary image methods as CT and MRI were used: CT was used in 52.38% of the patients and MRI was used as complementary to CT images in one case.[25] Besides, 66.6% of the patients underwent surgery, or drainage in one case, and parasite infection was confirmed by pathological analysis. Serology was performed in all patients. Prior to serology, sera were heat-inactivated at 56°C for 30 minutes. Absence of CE in control donors was confirmed by abdominal ultrasound and chest X-ray. Serological test for CE by HF-Western blotting (WB) and HF-ELISA were performed using an "in-house" G1 parasite preparation,[26] and Double Diffusion Arc5 (DD5) was run at the Centro de Zoonosis, in Azul.

2.2 | Experimental infection of sheep and collection of sheep serum samples

Serum samples were obtained from an EG95 vaccination study.[27] Briefly, *Echinococcus granulosus* eggs were collected from infected dog stool, in an endemic zone of the mountain ranges of Chubut, Argentina, after arecoline treatment. The isolated gravid *E granulosus* G1 was confirmed by macroscopic and genotype evaluations. Seven 4-year-old Merino sheep from a CE-free zone (Península Valdés) were then challenged by means of oral administration of ~ 2000 eggs of *E granulosus* per sheep. The sheep were slaughtered after 700 days, and a detailed inspection showed an average of 52 (13-125) and 49 (12-124) viable hepatic and pulmonary hydatid cysts per sheep, respectively, and one cyst in the spleen of one of the sheep. Five serum control samples were collected from noninfected and nonvaccinated animals, confirmed by the absence of visible cysts in their organs. Sera were heat-inactivated at 56°C for 30 minutes prior to testing each of them individually.

2.3 | EGPE cell culture and protein lysate

As mentioned above, EGPE is a cell line obtained from bovine *E granulosus* protoscoleces genotyped from its mitochondrial DCO1 sequence, maintained in our laboratory.[22] Extracts were

prepared from passages 35 to 40 and preserved in liquid nitrogen. Cells were then thawed, expanded and grown in vitro for 7 and 20 days, which corresponded to the "short" and "long" stages, respectively. Cells were aspirated from culture plates, pelleted and washed five times with DPBS. Pellets were incubated in lysis buffer containing 8 mmol/L CHAPS (MP Biomedicals), 10 mmol/L Tris (Sigma-Aldrich)–HCl (Anedra), pH 8, 2 mmol/L EDTA (Merck), 0.1% B-mercaptoethanol (MP Biomedicals) and 1/100 protease inhibitor cocktail (Sigma-Aldrich), at 4°C for 2 hours. Samples were then frozen and thawed three times and spun down at 10 000 g. Proteins were quantified by Bradford (Bio-Rad) and stored at −20°C until use.

2.4 | Protein extraction from hydatid fluid

Hydatid fluid from *E granulosus* G1, was collected in 10 mmol/L EDTA (Merck) and 3.7 g/L HEPES (Sigma-Aldrich) pH 7.4-8.0, concentrated in a 3K cut-off membrane concentrator (Pierce, Thermo Scientific) and then processed in lysis buffer as described above.

2.5 | Serology procedures

2.5.1 | Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and Western blotting

Proteins from EGPE cells (18 µg/lane), HF (10 µg/lane) and standard molecular weight markers (10-250 kDa, Bio-Rad or Genbiotech) were loaded and separated under reducing conditions in 15% mini gels by SDS-PAGE. The proteins were transferred to nitrocellulose membrane (Bio-Rad) for 1.30 hours at 300 mA, and the membrane was stained with red Ponceau and cut into individual strips. Nonspecific binding was blocked with 5% nonfat milk and 0.05% Tween-20 (Sigma-Aldrich) in TBS (blocking buffer) for 1 hours at room temperature. Each strip was then probed for 2 hours at room temperature with sera or plasma (diluted 1:125 in blocking buffer) or anti-EG, mouse monoclonal IgG1 (EHG clone, Cat # SC-51875, Santa Cruz Biotechnology Inc) diluted 1:300 in blocking buffer, and then washed three times with TBS-0.05% Tween-20. Alkaline phosphatase-goat anti-human IgG (1:10 000, Cat # A9544, Sigma-Aldrich), rabbit anti-sheep (1:5000, Cat # SC-2773, Santa Cruz Biotechnology Inc) or goat anti-mouse IgG (1:5000, Cat # A3562, Sigma-Aldrich) were diluted in the blocking buffer, added to the corresponding strip and incubated for 1 hours. The samples were washed three times with TBS-0.05% Tween-20, and the reaction was visualized using BCIP/NBT substrate solution (Sigma-Aldrich). The bands were analysed using the GelAnalyzer software.

2.5.2 | Indirect ELISA

High binding ELISA plates were coated with 1 µg/well[26] of HF *E granulosus* G1 protein concentrate in 0.25 mol/L carbonate/bicarbonate buffer (pH 9.6) at 4°C overnight. Afterwards, the plate was washed and incubated with blocking buffer (PBS, 0.075% Tween-20 and 5% nonfat milk) for 1 hours at 37°C. Serum samples from CE patients and their controls as well as control human serum (Cat # H4522, Sigma-Aldrich) were diluted in blocking buffer (1:125) and incubated at 37°C for 1 hours. After five washes with PBST (PBS and 0.075% Tween-20), peroxidase-conjugated anti-human IgG (Cat # A0170, Sigma-Aldrich) diluted 1:30 000 in blocking buffer, was added and incubated at 37°C for 1 hours. The peroxidase reaction was visualized after the addition of 100 µL of the TMB/well for 30 minutes, after thorough washing (eight times) with PBST, stopped with 50 µL of H_2SO_4 0.5 mol/L and then read at an optical density (OD) of 450 nm on a Glomax multifunction spectrofluorometer (Promega). The cut-off point was two standard deviations above the average of the OD values obtained with commercial healthy human sera[26] (Cat # H4522, Sigma-Aldrich).

2.6 | Proteomic analysis

EGPE protein extracts were run in 15% SDS-PAGE, fixed overnight in 30%,v/v, ethanol and 2%,v/v, phosphoric acid, then washed three times with distilled water and dyed with colloidal Coomassie blue solution in water (18% methanol, 170 g/L ammonium sulphate, 2% phosphoric acid and 0.5 g/L Coomassie Blue G-250). After 2 days, excess dye was removed with several washes in distilled water. The 70-kDa protein band was cut from the gel and processed for mass spectrometry analysis at the MS service of the Centro de Estudios Químicos y Biológicos por Espectrometría de Masa (CEQUIBIEM) of the Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, Argentina. Processing consisted in trypsin digestion and the masses of recovered peptides were determined in a Q-Exactive mass spectrometer (Thermo Scientific) and analysed with Proteome Discoverer (Thermo Scientific, Version 1.4) with a precursor-ion mass tolerance of 10 ppm and fragmentation mass tolerance of 0.05 Da. The peptide sequences were compared against a UniProt database for *E granulosus*.

2.7 | AgB2 subunit PCR

DNA from EGPE cells, G1 protoscoleces and the HT29 cell line were obtained by the phenol/chloroform method. PCR reaction was carried out in 20 µL, final volume, reaction buffer (0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$) with 0.25 µmol/L AgB2F: 5′-TCTTGCTCTCGTGGCTTTCG-3′ and AgB2R: 5′-CCATGTGTGCTTTTGGCTCA-3′ primers, in the presence of 1U DNA polymerase (Taq Pegasus PB-L). DNA was denatured for 2 minutes at 94°C, annealed with the primers in 35 cycles of 30 seconds at 92°C, 30 seconds at 55°C, 30 seconds at 72°C and extended for 5 minutes at 72°C. A band of 130 nucleotides was recovered from a 1.5% agarose gel and sequenced at Instituto Malbrán (ANLIS).

2.8 | Mouse immunization

A mixture of proteins from EGPE extracts from the "short" and "long" stages was loaded and separated in a 15% gel by SDS-PAGE.

TABLE 1 Characteristics of the patients

Cyst organ localization	Habitat			WHO ultrasound classification				
	U	R	UR	CE1	CE2	CE3	CE4	ns
Liver	9	2	2	9	1	–	2	1
Lung	–	1	–	–	–	–	–	1
Liver—and others	2	3	–	–	–	1	3	1
Muscle	1	–	–	–	–	–	–	1
Abdomen	1	–	–	–	1	–	–	–

Note: Inhabitants are from urban (U) and rural areas (R). Cystic echinococcosis was staged by ultrasound. The disease was classified according to the WHO classification (CE 1 to 5). About 80.95% of the patients were staging. Patients that presented cyst with patterns not classified by WHO or localized in other organs were not staged (ns). All patients received albendazole and only 13 patients underwent cystectomy.

The gel was soaked in cold 0.1 mol/L KCl (Anedra) for 15 minutes, and a band containing a 70-kDa protein was cut. Slices of gel without protein were used as control. The gel fragments were homogenized in DPBS with a tissue grinder, left overnight in the rotator in a cold room and then spun down at 10 000 g. Three Balb/c male 2.5-month-old mice were then inoculated intraperitoneally with the supernatant, 6-10 μg of proteins or control solution on days 0 and 28. On day 39, mice were inoculated with 2.5 μg of proteins. Plasma samples obtained by a submandibular puncture on day 42 after the first inoculation were used to perform a WB for EGPE and HF antigen recognition.

2.9 | Sample discrimination and statistics

The bands detected by WB in CE patients' sera were distinguished from bands detected in the paired control in agreement with a match case-control design. The patients' sera were considered reactive when at least one of the band was recognized by the case serum and not by the matched control sample. In *E granulosus*-infected sheep, the WB band was considered positive when it was absent in all control sheep. Sensitivities were calculated considering the proportion of CE patients that were reactive to EGPE cells or HF protein extracts. The specificity in WB was calculated considering control samples that did not recognize any reactive bands. In ELISA, sensitivity was calculated considering the false negatives and specificity considering the false positives, obtained as described above. Statistical significances were calculated by Chi-square test with Yates' correction and Finney contingency 2 × 2 Table.

2.10 | Ethical statements

The human serum specimens were collected following approved protocols and procedures according to the Institutional Health Research Ethics Committee and approved by the Ethics Committee of the "Universidad Abierta Interamericana," number 01011. Animal experiments were performed according to the National Bioterial System (ID850-41) of the Argentine Ministry of Science, Technology and Productive Innovation (MinCyT) in compliance with the National Administration for Food, Drugs and Medical Technology (ANMAT) Provision 6344/96 and is under the control of the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the School of Veterinary Science of the University of Buenos Aires, Argentina, which ensures that all activities involving animals are performed ensuring the health and welfare of all the animals and within the framework of international standards of ethics and biosafety. Mouse handling and sample collection protocols and procedures were approved by the Ethics Committee of the "Universidad Abierta Interamericana," number 01011. The experiment in sheep was approved by the Ethics Committee of the Instituto de Ciencia y Tecnología Dr Cesar Milstein (number 123/17).

3 | RESULTS

The study performed in CE patients had a case-control design matched by habitat (rural or urban; Table 1). The results of the serological test were found to be independent of the patient's habitat and the cyst localization, and most of the patients had the parasite in the liver.

The sera from CE patients and experimentally infected sheep were shown to recognize SDS-PAGE-separated proteins in the EGPE extracts (Figure 1 A and B), whereas the sera from patients also recognized the HF extract (Figure 2). Figure 1A and Figure 2 show sera antigen recognition by the same human serum samples. Commercial monoclonal anti-EHG antibody reacted with EGPE and HF protein extracts, protein bands 46 and 69 kDa.

3.1 | Proteins from EGPE are recognized by sera from CE patients

To evaluate the serum reactivity, proteins from the "short" and "long" stages of EGPE were resolved by SDS-PAGE and subsequently transferred to membranes. Bands of WBs performed with sera from CE patients spanned from 12 to 94 kDa. The proteins from the "short" stage confirmed the diagnosis in 18 out of 21 patients (calculated sensitivity of 85.7%), whereas that from the "long" stage confirmed the diagnosis in 16 out of 21 patients (calculated sensitivity of 76.2%). Furthermore, the combined EGPE-antigen reactivity

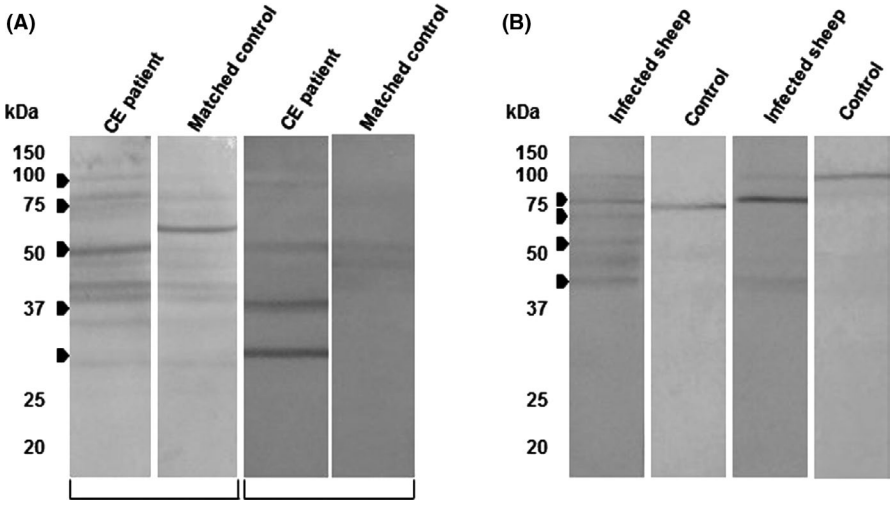

FIGURE 1 Representative examples of EGPE antigen recognition by Western blot: Sera from Cystic echinococcosis (CE) human patients (A) and sera from CE-infected sheep (B) recognize antigens from EGPE cell protein extracts from "long." Arrows indicate positive CE serum reactivity. Parentheses indicate the sample and its paired-matched control

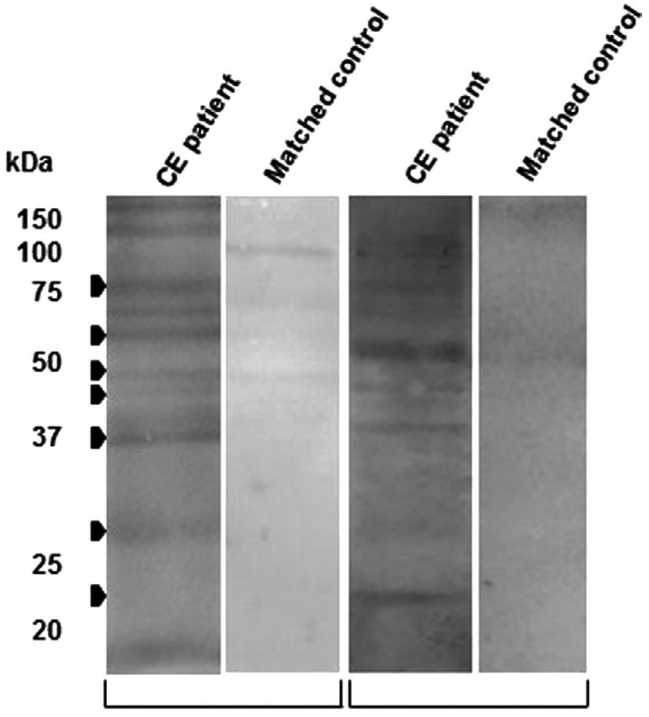

FIGURE 2 Representative examples of HF antigen recognition by Western blot: Sera from Cystic echinococcosis (CE) human patients recognize antigens from HF protein extracts. Arrows indicate positive CE serum reactivity. Parentheses indicate the sample and its paired-matched control

of the 21 sera showed 100% sensitivity. About 95.24% of the samples reacted to bands spanning between 37 and 60 kDa (specificity of 23.81%), and 71.4% of the samples reacted to the 37-46 kDa band (specificity of 57.14%). A statistical difference was found between the HF-WB and EGPE-WB, whose sensitivity was of 71.4% and 100%, respectively ($P < .05$, Chi-square test Yates' correction). No statistical difference was found in specificity between HF-WB (19.05%) and EGPE-WB (14.28%).

The distribution of the EGPE proteins from the "short" and "long" stages reacting with all human CE serum samples also revealed significant differences in the recognition of three of the protein bands corresponding to molecular weights of 37-46, 47-52 and 53-55 kDa ($P < .05$; Figure 3).

The indirect HF-ELISA test resulted in 12 false positives and 5 false negatives. However, HF-ELISA gave 76.2% of positivity in CE patients, a result that was not significantly different from that obtained with HF-WB and EGPE-WB (Chi-square test Yates' correction).

DD5 assessed in CE patients was positive in 71.4% of the samples, with a significantly lower detection when compared to EGPE-WB ($P < .05$, Chi-square test Yates' correction).

3.2 | Proteins from EGPE are recognized by sera from infected sheep

Sera from sheep challenged with *E granulosus* G1 eggs reacted with EGPE extracts, as seen by WB (Figure 1B), and all of them recognized protein bands from the "short" and "long" stages, with significant differences ($P < .05$, Finney test; Figure 4). In addition, the protein band recognized by all serum samples from CE-infected sheep corresponded to the 44-48 kDa from the "long" stage, whereas the protein band of 56-64 kDa was equally recognized in both EGPE extracts. Only one sample, belonging to a sheep with multiple cysts in the liver and one cyst in the spleen, recognized fewer protein bands by EGPE-WB (75-83 and 44-48 kDa). Finally, no correlation was found between WB-EGPE results and the number of cysts.

3.3 | EGPE expresses AgB2

The proteomic analysis of the ~70 kDa band allowed identifying the AgB2 subunit (C1KBK7). The sequence for the AgB2 gene was confirmed by PCR in EGPE cells and was 100% homologous to that of AgB2 from *E granulosus* G1 protoscoleces DNA (CACGTCT CCTTCTCTTGTCTCCACACCTCATTTTCACATTTGTCACCTCCC TTTTAGTAAAGATGAGCCAAAAGCACACATGG).

The hyperimmune plasma from mice inoculated with this identified band recognized three protein bands of 60 to 90 kDa from

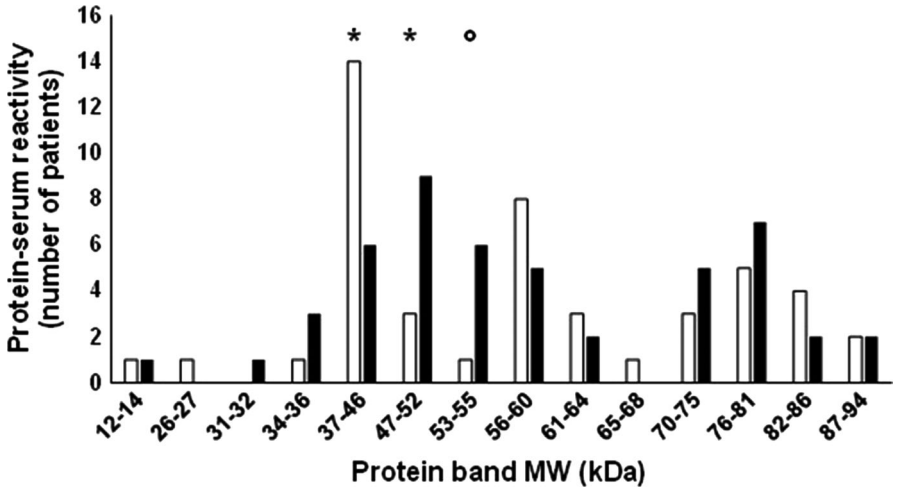

FIGURE 3 Cystic echinococcosis (CE) human serum reactivity to EGPE protein extracts: The *X*-axis represents the molecular weight of the protein bands, whereas the *Y*-axis represents the frequency of EGPE protein band recognition by sera. Significant differences were found between "short" (□) and "long" (■) EGPE cell culture extracts (n = 21 patients, in triplicate). A protein band was considered positive when it was discriminated from the paired-matched control. The distribution of protein bands was analysed with the GelAnalyzer program. *: $P < .05$ (Chi-square test) and °: $P < .05$ (Finney contingency 2×2 table)

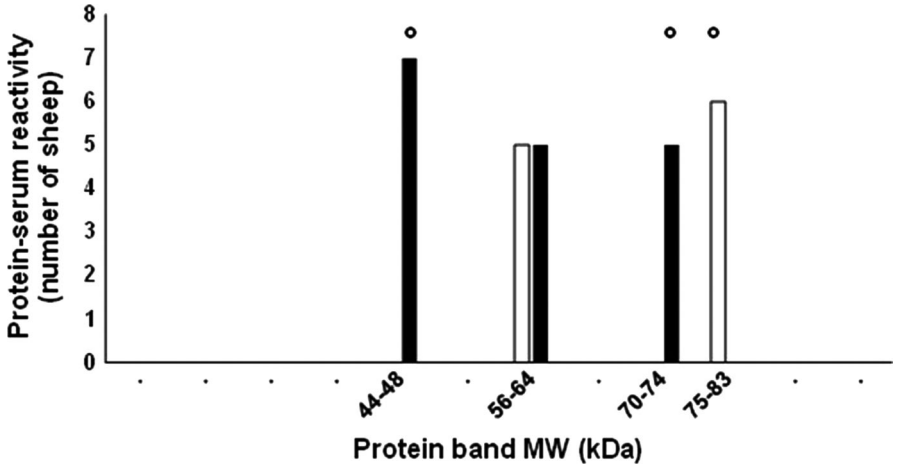

FIGURE 4 Reactivity of the sera from CE experimentally infected sheep to EGPE protein extracts: The *X*-axis represents the molecular weight of the protein bands, whereas the *Y*-axis represents the frequency of EGPE protein band recognition by sera (n = 7). Significant differences were found between "short" (□) and "long" (■) EGPE cell culture protein extracts. A protein band was considered positive when it was discriminated from control sera. The distribution of protein bands was analysed with the GelAnalyzer program. °$P < .05$ by Finney contingency 2×2 table

EGPE and HF antigens by WB (Figure 5). This allowed confirming the antigenic properties of the EGPE protein components.

4 | DISCUSSION

Protein extracts from two EGPE cultures of different growth periods, termed "short" and "long," were chosen to evaluate the positive reactivity of serum from CE patients and CE-infected sheep because EGPE cell morphology and membrane development change over the culture growth time.[22] In our matched case-control study, these EGPE extracts were 100% reactive with sera from CE patients by WB, whereas with the other assays performed, that is, HF-WB or ELISA and DD5, reactivity fluctuated between 71.4% and 76.2%. Western blotting (or immunoblotting) is consistently one of the most sensitive methods in the immunodiagnosis of CE patients,[28] whereas HF-ELISA typically displays a high frequency of false positives among the CE-free control samples. Regarding specificity, EGPE-WB did not improve the specificity of HF-WB.

In the present study, sera from CE patients reacted with EGPE proteins, spanning 12 to 94 kDa, showed 100% of sensitivity, while other authors considered the range spanning 6.5 to 239 kDa relevant in positive diagnosis.[29-31] Remarkably, as shown, considering bands from EGPE extracts between 37 and 60 kDa showed a positivity of 95.23%, consistent with CE disease. In addition, the bands detected within molecular weights 37-46 kDa showed 71.4% of sensitivity with the highest specificity (57.14%).

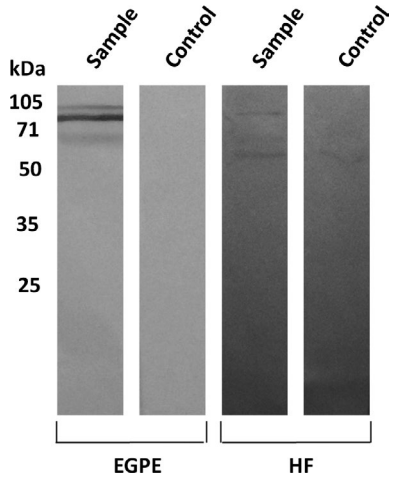

FIGURE 5 Hyperimmune plasma from immunized mice recognizes EGPE and HF antigens by Western Blot: Protein band identification in EGPE and HF protein extracts by hyperimmune plasma obtained from mice inoculated with the 70-kDa EGPE protein band. Plasma control was from mice injected with an elution solution

Consistent with the genome of *E granulosus* G1 genotype,[32] AgB2, which some authors considered as a hallmark for CE diagnosis,[24] was found in the EGPE 70 kDa band protein extract. Our results also demonstrated a positive reactivity of sera from experimentally infected sheep, in agreement with their humoral response,[33] thus validating the antigenic properties of EGPE protein extracts for the recognition of serum from CE intermediate hosts.

The differences observed in serum recognition of proteins from the "short" and "long" stages suggest that different antigenic proteins are expressed in EGPE over time, or that differences are a function of the immunological response elicited by the parasite as the disease evolves.[7,33,34] These findings support the need for further characterization of protein identity and diversity in each of the reactive bands.

Summarizing, EGPE cells express *E granulosus* sensu lato antigens recognized by sera from CE patients and experimentally infected sheep. This was here demonstrated by the differences in immunogenicity of the EGPE extracts from the "short" and "long" stages, as sources of different antigens for CE diagnosis. Despite the small population studied, the fact that this study was performed in a matched case-control design with patients from an endemic area of Argentina strengthens the findings. Furthermore, the sensitivity of the EGPE extracts was higher than that of the HF extracts assayed in the same conditions. More serum samples would help to find more discernible results using EGPE cell protein extracts, and the identification of proteins and their reactivity could improve the specificity of the EGPE-WB for serology.

Finally, EGPE is advantageous over fresh, variable metacestode extracts from the slaughterhouse, including HF. Cells are maintained and stored in nitrogen, then thawed and expanded before use and grown in axenic medium without any contamination from the host cells. Thus, the quality and growth of EGPE cells are controlled in the laboratory. Further studies are necessary to identify and characterize the proteins involved in the differential recognition of the "short" and "long" EGPE-antigens for the improvement of CE serology.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTIONS

AGF designed the study; AGF, JG, OJ and GC conceived the study; AM, MSBV, CH, MLG, and VP acquired the data; AGF and AM analysed and interpreted the data; AGF, AM and GC drafted the manuscript and revised it critically. All the authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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