BioChemMed

Technium BioChemMed Vol. 13, pp.64-71 (2025)
ISSN: 2734-7990
www.techniumscience.com

Determination of immune reactive proteins in Hydatid Cyst that isolated from sheep in Kerbala province- Iraq

Jihad Talib Obead[1], Nada Abdulhussein Alkafaji[2], Sajad Adnan Khudair[3], Ihsan Mohammed Sulbi[4]

1,4. Department of Microbiology and Parasitology, Collage of Veterinary medicine, University of Kerbala
2. College of Veterinary Medicine, University of Kerbala.
3. Department of Veterinary public health College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Abstract Hydatidosis is among the most common parasitic zoonotic diseases, not just in Iraq but globally. Worldwide, in domestic animals and humans are at significant effect for unilocular hydatid disease, This is brought on by the parasite *E. granulosus*. The zoonotic parasitic disease known as cystic echinococcosis, brought on by the tapeworm *E. granulosus*. Through development of parasite, dogs and carnivores serve as the final hosts, whereas herbivores and omnivores, such as humans, serve as intermediated hosts, where the metacestode (larval stage) grows in the organs, primarily the lungs and liver. Although sheep have lately drawn increased attention as *E. granulosus* infection reservoirs among intermediate hosts, the understanding of the localized inflammatory responses associated with liver cystic echinococcosis is limited *Echinococcus granulosus* larvae, known as hydatid cysts, are frequently seen in Iraqi sheep and goats that have been killed. Our study's objective was to identify immune reactive proteins in hydatid cysts extracted from infected sheep's liver. In this investigation, samples of liver from sheep slaughtered at the Karbala Abattoir in Iraq were used to acquire fluid from Hydatid cysts (N = 25 samples). The SDS-PAGE technique (Sodium Dodecy Sulfate-Polyacrelamide Gel Electrophoresis) was used to analyze different proteins in the fluids extracted from cysts in order to identify reactive proteins. The fluid antigens' SDS PAGE results showed 85 kDa, 70 kDa, 46 kDa, 34 kDa, 23 kDa, 9 kDa, 4 kDa, and 2 kDa bands for hydatid cysts. Conclusion of current study, improve our understanding of immune responses to cystic Echinococcosis in intermediate hosts, identify disease progression markers, and offer initial insights for future research on hydatid cyst immune reactions

Keywords: Hydatid cyst, SDS -PAGE, *Echinococcus granulosus*

Introduction

The tapeworm *Echinococcus granulosus* larval stages (metacestodes) cause of the zoonotic parasitic disease known called as echinococcosis (CE) **(Jawad *etal*., 2018; Atmaca *et al*., 2019; Bosco *et al*., 2021).** The adult cestode generates eggs that carry the infectious oncosphere and lives in the small intestine of carnivore-definitive hosts, such as dogs and other canids **(Jiménez *et al*., 2020).** After the intermediate host consumes oncospheres, the metacestode grows as a fluid-filled cyst in the viscera, primarily the liver and lungs. Humans can become accidentally infected as intermediate hosts mostly through contaminated food or drink or direct contact with infected dog excrement **(Beigh *etal*., 2017;**

Technium BioChemMed Vol. 13, pp.64-71 (2025)
ISSN: 2734-7990
www.techniumscience.com

Atmaca. 2022). The structure of an *Echinococcus granulosus* cyst is extremely complex and comprises Both a host-derived (adventitia) and parasite (hydatid) components **(Abo-Aziza *et al*., 2020).** There are two layers in the parasite-derived parts: the laminated layer, which is an exterior acellular layer that generates daughter vesicles, protoscoleces (PSC), brood capsules, and hydatid cyst fluid (HCF); and the germinal layer, which is an inner nucleated layer. Host-generated fibrous capsules surround both the laminated and germinal layers **(Atmaca. 2022).** Hydatid cysts can be categorized as either sterile or fertile based on whether protoscoleces are present in the walls of the cyst or inside the cysts. Pre-encystment and post-encystment are the two stages of the immune response to an E. granulosus infection, according to conventional wisdom. The way the laminated layer develops around the evolving infectious oncospheres and intermediate hosts varies between these phases. A The type immune response is triggered by the early-establishment-phase cysts, and it may be responsible for eradicating most infectious parasites and producing high levels of protection against a second challenge **(Rickard and Williams.1982; Tamarozzi *etal*.,2016).** The condition is only discovered after the infection has worsened since its symptoms have been gradually appearing, this cyst is primarily seen in the liver and lung, although it may also be found in the kidneys, bone marrow, spleen, heart, and brain **(Liu *et al*., 2018).** For hexacanth embryos in circulation, the hepatic capillary acts as the first capillary filter, which gradually grow into hydatid cysts in the liver, however, hydatid cysts can occur anywhere in the intermediate host body (human and animal), therefore, until the intermediate animal hosts reach a sufficient volume to start exhibiting symptoms, the illness stays asymptomatic **(Eckert *et al*., 2001; Mnati *et al*., 2020).** Because of the host's immune system and the gradual growth and development of cysts, CE can go unnoticed for many years **(Zhang *et al*., 2000; Siracusano *et al*., 2012a).** Cysts may eventually put pressure on surrounding tissues, causing pain and discomfort in the abdomen, depending on their size and location **(Siracusano *et al*., 2009; Gottstein, 2000; Siracusano *et al*., 2012b).** Cysts in the liver can compress bile ducts, causing obstruction that may manifest as obstructive jaundice, stomach pain, anorexia, and pruritus, whereas cysts in the lungs can irritate the membranes, causing hemoptysis, pleuritic chest discomfort, persistent cough, and dyspnea **(Gottstein. 2000; Symeonidis *etal*., 2013).** Immunologic symptoms resulting from increased immunoglobulin (Ig) levels might be caused by cyst rupture or leaking. Anaphylactic shock, hives, and pruritus are among the allergic responses linked to IgE, IgG2, and IgG4 **(Siracusano *et al*., 2012a).** Using sodium dodecy sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), proteins originating from Taenia hydatigena, Taenia saginata, Echinococcus granulosus, and Taenia ovis cysts were isolated. The parasite uses variety of tactics, including antigenic mimicry, antigenic depletion, and antigenic variation, to try to evade the host immune response and weaken the host defenses. It appears that this helminth's immune response regulation benefits both the parasites and the human host **(McSorley and Maizels .2012).**

Materials and methods

1- Collection of Hydatid cysts

In the slaughterhouses, post-mortem examinations were performed by ocular inspection, Each carcass and its internal organs are palpated and carefully cut, especially the liver and lung, after macroscopic organ examination and palpation. A cooling box (Veterinary College in Kerbalaa/Parasitology laboratory) was used to transport the hydatid cyst samples—roughly 50 cysts infected with cystic hydatidosis of the sheep carcasses produced in the abattoirs (Kerbala province)—to the laboratory for analysis **(Al-hussainy.2015).** Only protoscoleces (PSCs)-containing viable cysts were chosen; cysts that were calcified or pierced were not. First, Cystic fluid was extracted from a sheep host in order to

Technium BioChemMed Vol. 13, pp.64-71 (2025)
ISSN: 2734-7990
www.techniumscience.com

prepare antigen. Next, a sterile 20 mL syringe was used to aspirate the hydatid cyst fluid (HCF), which was then collected in 50 mL sterile tubes. Finally, Protoscolices were removed from the hydatid fluid from the sheep liver fluid by centrifuging it at 10,000xg for 30 minutes at +4ºC. Filtration of the supernatant was done using a 0.4 mm membrane filter. Dialyzing the filtered solution against pure water at +4ºC for 36 hours **(Hajizadeh *etal*., 2024**).

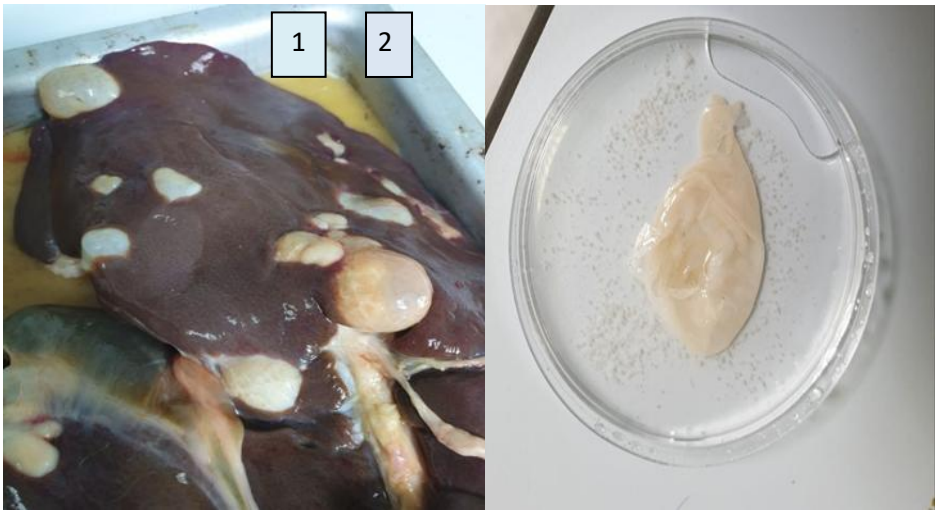

Figure (1). A- revealed that liver with multiple hydatid cyst. **B-** showed that fluid with protoscoleces have been collected from hydatid cyst

2- SDS-PAGE is used to detect fluids' reactive peptides.

A. The protein precipitation methodology for A-Trichloroactetic acid (TCA) was developed by **(Koontz. 2014).**

B. The Resolving and Stacking Gels recipe, which calls for 5 milliliters of 10% polyacrylamide Gel Mix **(Hashim et al., 2019).**

C. Gel staining technique in accordance with C-Silver staining **(Blum *et al*., 1987).**

Results

SDS-PAGE is used to determine the reactive proteins in hydatid cyst fluid.

The Protein Ladder with molecular weights 6, 12, 17, 20, 24, 35, 49, 64, 76, and 100 KDa used to analyze Cystic fluid's active proteins extracted from the viscera of sheep infected by Hydatid cyst use the SDS-PAGE technique. the outcomes Hydatid cyst fluid's reactive proteins were identified using SDS-PAGE.The Hydatid cyst fluid included four distinct protein bands, ranging in size from 85 to 1 kDa as follows: 85, 70, 46, 34, 23, 9, 4, 2 and 1 kDa **(Table 1)** and **(Fig. 2)**

Technium BioChemMed Vol. 13, pp.64-71 (2025)
ISSN: 2734-7990
www.techniumscience.com

(Table.1). shown the Hydatid cyst protein bands after they were separated using SDS-PAGE.

Band	Molecular weight KD of Hydatid cyst
1	85
2	70
3	46
4	34
5	23
6	9
7	4
8	2
9	1

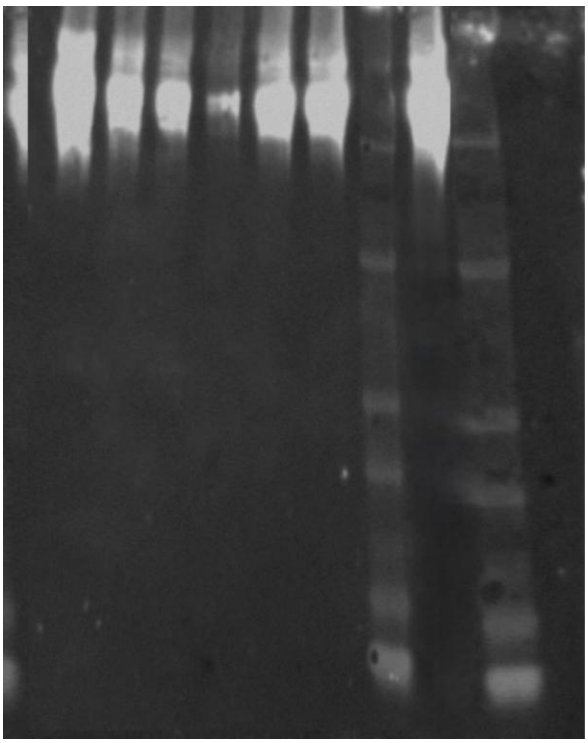

Figure (2):. SDS-PAGE resolution of the polypeptide profiles of the cystic fluid generated from Hydatid cysts, followed by silver staining of the sample (1) Hydatid cyst fluid (2) The BLUslf prestained protein

Technium BioChemMed Vol. 13, pp.64-71 (2025)
ISSN: 2734-7990
www.techniumscience.com

Discussion

The present study showed that the protein band appeared were nine clearly visible protein bands in the hydatid cyst fluid , and they ranged in size from 85 to 1 KDa. as follows: 85, 70, 46, 34, 23, 9, 4, 2 and 1 kDa. In the examined cestode, certain protein bands were quite visible and showed up in large numbers, while others showed up in very little amounts and were faint. The 85, 70, and 46-kDa proteins were found in every investigated cestode species, which is noteworthy when compared to prior studies of a similar nature **(Abuseir *et al.*2018).** This might also account for the fact that some samples had smaller proteins that showed up on SDS-PAGE, whereas other samples had nonexistent or extremely weak tiny proteins. Furthermore, certain cestode infections in the animal body occur due to the presence of certain proteins in distinct places **(Miquel *et al*., 2015).** Protein extracts of hydatid cyst fluid (HCF) from Saudi Arabian sheep and camels that were infected were subjected to the SDS-PAGE method in order to compare the diagnostic values of the antigenic fractions. According to the findings, the HCF preparations from sheep and camels had 11 separate and significant antigenic fractions (110–8 kDa) **(Al-Olayan and Helmy, 2012).** SDS-PAGE examination revealed one band of 72 kDa for purified antigen and four bands of 72, 64, 48, and 24 kDa for crude antigen **(Siddartha *et al*., 2022).** A research found that the hydatid cyst fluid's protein profile showed nine bands with a molecular weight of 8–205 kDa, and that the HCFA antigens were separated using 12% SDSPAGE. This was done in order to identify the particular protein of cystic echinococcosis and to examine various regions of the ovine bladder worm **(Jeyathilakan *et al*., 2021).** Since these parasites had a common ancestor at the time of their development, cross-reactivity is a characteristic shared by both phylogenetically related and unrelated parasites. It results from common antigens being widely distributed, indicating that antigenic continuity is the norm rather than the exception **(El-Moghazy and Abdel-Rahman .2012).** Efforts to increase the immune response by using Hydatid cyst fluid Ag rely on the parasite protein. According to **Abuseir *et al*. (2018)**, the antigen complexity is a unique phenomenon of immunological cross-reactivity between parasites of different species. It is also essential for the development of cross-protective immunity in hosts against several parasitic infections, because parasites' antigenic structures are so similar, it is frequently possible to use a diagnostic antigen from one species to protect against another. **El-Moghazy and Abdel-Rahman (2012)** concur that in some circumstances, employing a cross-reactive antigen to guard against many infestations may be helpful. In another investigation, hydatid cyst fluid from humans, pigs, goats, and sheep had 15 protein fractions with molecular weights ranging from 8 to 116 kDa. Additionally, antibody responses were developed against 12 protein fractions of sheep hydatid fluid in humans infected with hydatidosis. These findings are consistent with the findings of **Kanwar *et al*. (1992) and Burgu *et al*. (2000).** Heath and Lawrence used SDS-PAGE to extract bands 23 and 25 kDa from Echinococcus granulosus egg onchospheres, and they then examined these proteins on sheep, the sera of these sheep generated specific antibodies that lyse oncospheres **(Heath and Lawrence, 1996).** Many variables, such as the various animal species from which the antigen was generated and strain differences that have been documented in the same animal species within a nation, might be responsible for the alterations in the protein band pattern. According to **Ahmad *et al*. (2001) and Jeyathilakan *et al*. (2021),** it also relies on the various purification techniques used, antigen manufacturing techniques, and storage techniques, the dissociation of bladder worms' very high molecular weight protein complexes into two or more subunits of lesser size under decreasing circumstances is another explanation for this, some samples had smaller proteins that showed up on SDS-PAGE, while in other samples, these proteins were either nonexistent or extremely

weak. Additionally, the establishment of metacestode infestations in the animal body involves distinct protein sites, which is consistent with **McManus (2014)**

Conclusion

This information might improve our knowledge of the immune responses to cystic Echinococcosis (CE) in intermediate hosts and help identify immunological markers for distinguishing the course of the disease. The results may offer initial information for upcoming research on the immune reactions linked to hydatid cysts.

References

[1] **Abo-Aziza, F.A.M., Hendawy, S.H.M., Oda, S.S., Aboelsoued, D., El Shanawany, E.E.(2020).** Cell-mediated and humoral immune profile to hydatidosis among naturally infected farm animals. Vet. World 2020, 13, 214–221.

[2] **Abuseir, S .,Nagal-Kohl, U and Strube, C.(2018).** Protein profile of the cysts of Taenia hydatigenia, Taenia saginata, Echinococcus granulousus and Taenia ovis.HVM Biofleux .10 (4):175-179

[3] **Ahmad, G., Nizami, W.A and Saifullah, M.K. (2001).** Analysis of potential antigens of protoscolices isolated from pulmonary and hepatic hydatid cysts of Bubalus bubalis. Comparative Immunology, Microbiology and Infectious Diseases. 24: 91-101

[4] **Al-hussainy, M.H.(2015).** Efficacy and immunopathological role of bile salts against hydatidosis comparing with antigen B in experimentally infected mice. M. Sc Thesis in veterinary medicine, collages of Baghdad University, Iraq.2015

[5] **Al-Olayan, E. M and Helmy, H. (2012).** Diagnostic value of different antigenic fractions of hydatid cyst fluid from camel and sheep in Kingdom of Saudi Arabia. Journal of Saudi Chemical Society. Vol 16, Issue 2, PP. 203-207

[6] **Atmaca, H.T.(2022).** Determination of macrophage types by immunohistochemical methods in the local immune response to liver hydatid cysts in sheep. Acta Trop. 229, 106364.

[7] **Atmaca, H.T., Gazyagci, A.N., Terzi, O.S., Sumer, T.(2019).** Role of stellate cells in hepatic echinococcosis in cattle. J. Parasit. Dis. 43, 576–582.

[8] **Beigh, A.B., Darzi, M.M., Bashir, S., Kashani, B., Shah, A., Shah, S.A.(2017).** Gross and histopathological alterations associated with cystic echinococcosis in small ruminants. J. Parasit. Dis.41, 1028–1033

[9] **Bosco, A., Alves, L.C., Cociancic, P., Amadesi, A., Pepe, P., Morgoglione, M.E., Maurelli, M.P., Ferrer-Miranda, E., Santoro, K.R., Nascimento Ramos, R.A., et al.(2021).** Epidemiology and spatial distribution of Echinococcus granulosus in sheep and goats slaughtered in a hyperendemic European Mediterranean area. Parasites Vectors. 14, 421.

[10] **Burgu, A, Doğanay, A, Gönenç, B, Sarimehmetoğlu, h. O and kalinbacak, F (2000).** Analysis of Fluids of Hydatid Cysts from Sheep by SDS-PAGE, and Determination of Specific Antigens in Protein Structure by Western Blotting. Turkish Journal of Veterinary & Animal Sciences: Vol. 24: No. 5.pp.493-500.

[11] **Eckert, J., Deplazes, P., Craig, P.S., Gemmell, M. A, Heath, D., et al . Echinococcosis in animals: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX,**

Pawlowski Z (eds.) WHO/OIE manual on Echinococcosis in humans and animals: a public health problem of global concern, Paris: 2001;72–99

[12] **El-Moghazy, F.M and Abdel-Rahman, E. H. (2012).** Cross-Reaction as a Common Phenomenon among Tissue Parasites in Farm Animals. Global Veterinaria;8(4):367-373.

[13] **Jawad, A. R., Sulbi, I., Jameel, Y., & Ali, H. (2018).** Molecular detection of Echinococcus granulosis from visceral organs of cattle abattoirs Kerbala province. J. Pure Appl. Microbiol, 12(2), 701-704

[14] **Gottstein, B. (2000).** Hydatid disease, major tropical syndromes by body system, Syst. Infect. 169 section

[15] **Hajizadeh, M., Jabbari, A., Spotin, A., Hejazian, S. S., Mikaeili Galeh, T., Hassannia, H., Sahlolbei, M., Pagheh, A. S., & Ahmadpour, E. (2024).** Modulatory Effects of Hydatid Cyst Fluid on a Mouse Model of Experimental Autoimmune Encephalomyelitis. Veterinary Sciences, 11(1), 34.

[16] **Heath, D.D. and Lawrence, S.B.(1996).** Antigenic polypeptides of Echinococcus granulosus oncospheres and definition of proctetive molecules, Parasite Immunol., , 18, 347-357.

[17] **Jeyathilakan, N; Abdul, B. S., Lalitha, J., Daniel, J. C and Dhinakarraj, G.(2021).** Immunochemical localization of cross-reactive proteins of bladderworms with special reference to detection of specific antigen in cystic echinococcosis of sheep. Ind. J. Vet. & Anim. Sci. Res., 50 (2) 37-47

[18] **Jiménez, M., Stoore, C., Hidalgo, C., Correa, F., Hernandez, M., Benavides, J., Ferreras, M.C., Saenz, L., Paredes, R.(2020).** Lymphocyte populations in the adventitial layer of hydatid cysts in cattle: Relationship with cyst fertility status and Fasciola hepatica co-infection. Vet. Pathol. 57, 108–114.

[19] **Kanwar, J. R., KaushÝk, S. P., Sawhney, I.M. S., Kamboj, M. S., Mehta, S. K. and VÝnayak, V. K. (1992)**. Specific antibodies in serum of patients with hydatidosis recognised by immunoblotting, J. Med. Microbiol. 36,46-51.

[20] **Liu, C., Xu, Y., Cadavid-Restrepo, A. M., Lou, Z., Yan, H., Li, L., Fu, B., Gray, D.J., Clements, A. A., Barnes, T. S., Williams, G. M., Jia, W., McManus, D. P., Yang, Y. (2018).** Estimating the prevalence of Echinococcus in domestic dogs in highly endemic for echinococcosis. Infectious Diseases of Poverty,7:77 18-458-8.

[21] **Mcmanus, D.P. (2014).** Immunodiagnosis of sheep infections with Echinococcus granulosus in 35 years where have we come? Parasite Immunology, 36:125- 130.

[22] **McSorley, H. J., Maizels, R. M.(2012).** Helminth infections and host immune regulation. Clin Microbiol Rev. 25(4):585-608.

[23] **Miquel, J., Khallaayoune, K., Azzouz-Maache, S., Pétavy, A. (2015).** Spermatological characteristics of the genus Taenia inferred from the ultrastructural study on Taenia hydatigena. Parasitol Res.114(1):201-208.

[24] **Mnati, I. M., Mutlak, B. H., & Abed, N. D. (2020).** Histological changes in liver tissue resulting from hydatid cyst infection: comparison between sheep and cattle in Iraq. Medico-legal Update, 20(1), 1205.

[25] **Rickard, M. D., Williams, J. F. (1982).** Hydatidosis/cysticercosis: Immune mechanisms and immunization against infection. Adv. Parasitol. 1982, 21, 229–296.

[26] **Siddartha, V. P., Jagadeesh, A ., Madhava , T. R, Chengalva, V. R and Swetha, C. S. (2022).** A Comparative Evaluation of Four Different Immunoassays in the Diagnosis of Cystic

Echinococcosis Using a Crude and Purified Hydatid Cyst Fluid Antigen. Journal of Acta Parasitologica. Vol 67, pp. 1667–1679.

[27] **Siracusano, A., Delunardo, F., Teggi, A., Ortona, E. (2012b).** Host-parasite relationship in cystic echinococcosis: an evolving story, Clin. Dev. Immunol. (2012) 639362

[28] **Siracusano, A., Teggi, A., Ortona, E. (2009).** Human cystic echinococcosis: old problems and new perspectives, Interdiscip Perspect Infect Dis 474368, https://doi.org/ 10.1155/2009/474368.

[29] **Siracusano, A., Delunardo, F., Teggi, A., Ortona, E.(2012a).** Cystic echinococcosis: aspects of immune response, immunopathogenesis and immune evasion from the human host, Endocr. Metab. Immune Disord. - Drug Targets. 12 , 16–23,

[30] **Symeonidis, N., Pavlidis, T., Baltatzis, M., Ballas, K., Psarras, K., Marakis, G. et al., (2013).** Complicated liver echinococcosis: 30 years of experience from an endemic area, Scand. J. Surg. 102 171–177

[31] **Tamarozzi, F., Mariconti, M., Neumayr, A., Brunetti, E. (2016).** The intermediate host immune response in cystic echinococcosis. Parasite Immunol. 2016, 38, 170–181.

[32] **Zhang, W., Ross, A., McManus, D.(2000).** Mechanisms of immunity in hydatid disease: implications for vaccine development, J. Immunol. 181 (2000) 6679–6685,