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Evaluation of Hydatid Cyst Antigen for Serological Diagnosis

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Abstract

Background: Hydatidosis, a chronic zoonotic disease, has a distribution worldwide and is caused by the larval stage of the Echinococcus helminth. The Dot-ELISA test can diagnose hydatidosis quickly and accurately. Additionally, unlike other hydatid disease tests now used, this quick and affordable enzyme immunoassay is very serum-conservative and antigen-conservative, needing just nanogram levels of parasite antigen.

Methods: In the present cross-sectional study, crude and B antigens of hydatid cyst fluid were obtained to diagnose human hydatidosis using CIEP (Counter Immunoelectrophoresis), ELISA (Enzyme-linked Immuno Sorbent assay), and Dot- ELISA (Dot Enzyme linked Immuno Sorbent Assay) methods. Infected liver with a hydatid cyst was collected from Tehran's slaughterhouses to prepare cyst fluid in different stages. After extracting and purifying the Cyst fluid, it is centrifuged at 4ºc, then prepared to concentrate. The study also included sera from hydatidosis (n=60), samples of helminth parasites (n=55), fascioliasis (n=35), toxocariasis (n=20) and negative control (n=35) were tested by CIEP (Counter Immunoelectrophoresis), ELISA (Enzyme-linked Immune Sorbent assay), and Dot- ELISA (Dot Enzyme linked Immuno Sorbent Assay) methods. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows release 25.0 (SPSS Inc., Chicago, IL, USA).

Results: Crude antigen of hydatid cyst showed a specificity of 76.7%, a sensitivity of 93.3% using the ELISA method, and B antigen showed a specificity of 96.7% and sensitivity of 88.3% using the same method.
The crude antigen of the hydatid cyst exhibited a specificity of 68.9% and a sensitivity of 86.7% using CIEP. The B antigen showed a specificity of 87.8% and sensitivity of 83.3% using the same method.
The crude antigen of hydatid cyst having serum dilution at 1:800 exhibited a specificity of 83.3% and sensitivity of 100% using the Dot-ELISA method and B antigen having serum dilution at 1:800 serum showed a specificity of 100% and sensitivity of 98.3% using the same method. The results of this finding showed that B antigen has the maximum specificity to diagnose hydatid test using the Dot-ELISA method.

Conclusion: Hydatid cysts present with varied symptomatology. History of exposure to infected animals may not be present. A high degree of clinical suspicion combined with meticulous history and clinical examination supported by laboratory investigations are required for its diagnosis. The Dot-ELISA system with native antigen B is a viable approach for the immunodiagnosis of human hydatidosis that is preferred to infection.

Keywords: Hydatidosis, Antigen, ELISA, Dot ELISA, Serologic Diagnosis.

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Introduction

Hydatidosis is one of the most important zoonotic diseases, which has a cosmopolitan distribution worldwide as well as Iran, and it may give rise to economic and hygienic damage in various countries throughout the world as well as Iran every year. Humans are the intermediate hosts of this parasite, which can be infected by the larval stage (1, 2). The prevalence of hydatidosis has been reported to cover almost the whole part of Asia, the main

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↑What is "already known" in this topic:
Due to the overpopulation of domestic dogs in the nation, the rise of diseases are associated with them, and a general lack of knowledge among people regarding how to properly care for dogs is seen.

→What this article adds:
It is imperative to provide medical diagnostics facilities with quick, simple, and affordable diagnostic procedures.

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part of Africa and America, and large regions in Australia and Europe (3, 4). The parasite not only infects the liver and lungs but also infects other organs (1, 5).

Clinical diagnosis and interpretation of hydatid cyst, which is mostly based on radiological images, sonography, or C.T.Scan, may be difficult. Parasitological diagnosis of the disease is rarely possible due to the tissue location of cysts. Thus, serologic methods are extremely useful in diagnosing hydatidosis. Almost all current serologic tests, such as CFT (Complement fixation test), IHA (Indirect hemagglutination), LA (latex agglutination), IEP (ImmunoElectroPhoresis), CIEP (Counter Immunoelectrophoresis), ELISA (Enzyme-linked Immuno Sorbent assay), from the beginning till today has been used to diagnose hydatidosis and recently in addition to ELISA, Dot- ELISA test (Dot Enzyme linked Immuno Sorbent Assay) also has been added seriously to the serologic diagnosis of hydatidosis (6-9).

The serologic evaluation of the antigens has revealed different results. These differences are not only related to the nature and purity of the antigen but also to the difference in the characteristics of the studied population of patients (10, 11). However, there is a need to evaluate such tests in different countries and regions based on native antigens, so diagnostic efficiency and value of each one will be recognized based on the present facilities.

Methods

The liver infected with a hydatid cyst was collected from Tehran slaughterhouses for 3 months to prepare cyst fluid. Infected human sera and healthy human sera were obtained to apply in the present study. Livers were then washed with tap water. Cyst fluid was carefully aspirated off and collected in conical dishes. Firstly, hydatid fluid was centrifuged at 1000 g for 20 minutes. After removal of the precipitate, clear fluid was collected and stored in the fridge in sterile dishes at 4°C (12,13).

After delipidation of hydatid fluid, it was dialyzed against distilled water at 4°C overnight and then was lyophilized. The obtained powders were collected in suitable vials which were soon used as crude hydatid fluid antigen (CHFAg). The level of samples' protein content was measured by the Bradford method. Preliminary antigen separation is done by gel chromatography filteration (14-16).

10 ml crude hydatid fluid with a concentration of 8mg/ml was slowly placed on the surface of gel Sefadex G200 of chromatography having dimension 2.6×100 cm and its fractions collected to draw a curve of peaks. The fraction of each peak after filtering with Millipore 0.2 µ m and adding Sodium Azide (NaN3, 0.02% W/V) were stored in sterile vials at – 80°C.

Preparation and purification of Antigen B, which is one of the main antigens of hydatid fluid (AgB), was done according to Oriol & et al. method (17, 18). When the purification process was done, hydatid cyst fluid dialyzed against 0.005 µ acetate buffer, pH = 5 at 4°C. Then B antigen was isolated at 100 °C.

Counter Immunoelectrophoresis did antigen evaluation. Test was performed at various antigen concentrations and different human serum dilutions then results were recorded, accordingly (17, 18).

To determine the molecular weight of proteins, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed and results were recorded and compared, consequently.

To diagnose and titrate hydatid fluid crude and purified antigens, the ELISA test was used. To determine hydatidosis, the Dot- ELISA technique was also used (19, 20).

To evaluate purified and crude antigens, human sera were prepared as follows: Sixty sera from patients who had hydatid cyst disease, Fifty-five sera from patients with fascioliasis and toxocariasis, and Thirty-five sera from healthy control. However, the above-mentioned sera were poured into the small vials and frozen at -80°C.

Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows release 25.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as n (%). Also, sensitivity, specificity, positive predictive value (PPE, and negative predictive value (NPV) were calculated for various antigens and compared, separately (21).

Results

Patients' samples were analyzed at dilutions of 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 to determine the sensitivity and specificity of the Dot ELISA test.

For each antigen, Dot ELISA's sensitivity and specificity were evaluated. when sera at different dilutions and antigen B at a dilution of 1:800 were compared, the 98.3% sensitivity and 100% specificity were determined to be appropriate values in order to identify a hydatid cyst.

Several antigenic fractions were obtained by gel chromatography Sefadex G200.

In order to purify the antigen, the Oriol method was used and 3 mg antigen-B with relative purity was obtained. Then after dialyzing and pouring into the vials, applied as an antigen-B and overall, two types of obtained antigens were evaluated.

A-Crude antigen: Usually, hydatid cyst crude fluid contains, protein, lipid, carbohydrate, and 23 protein fractions. The Permeability of the host proteins into the cyst causes observation of Albumin and Glubin in hydatid fluid. Under SDS-PAGE, bands with a molecular weight of 8 ≥ 150 KD were observed in which the sharpest band with a molecular weight of 67 KD indicated the presence of an Albumin band.

B-Antigens group B: Bands having molecular weights of 12, 16, and 24 were revealed by SDS-PAGE.

Obtained antigens were studied by serologic methods. The crude antigen of hydatid fluid was detected by CIEP in 52 (86.6%) out of 60 sera infected with hydatidosis and 25 (31.1%) out of 90 control sera infected with fascioliasis, toxocariasis and healthy sera to be false positive and the maximum false positive was observed in 19

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(54.2%) sera infected with fascioliasis.

The purified antigen B was observed by the CIEP method to be false positive in 50 (83.3%) out of 60 sera infected with hydatidosis and 11 (12.2%) out of 90 control sera.

To determine the sensitivity of the CIEP method, coupled serial 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and 1:512 were prepared by using this method only at 1:128 dilution was positive.

Applied antigens that were used in this study had been purified the fraction of hydatid cyst antigen B, and the best- precipitated arch was obtained at 1:32 dilution.

The sensitivity and specificity of the CIEP for various antigens were compared and estimated, separately. Thus antigen P1G of crude hydatid fluid and P2G of antigen B showed a maximum sensitivity of 90 %.

To evaluate crude antigen of hydatid fluid using the ELISA method, 56 (93.3) out of 60 sera infected with hydatidosis and 2 (23.3%) out of 90 sera infected with fascioliasis, toxocariasis, and healthy people were detected to be false positive and among them, fascioliasis (n=16.46%) and toxocariasis (n=3.15%) were shown the maximum cases of false positive, respectively.

In the purified antigen B study, using the ELISA method 53 (88.3%) cases out of 60 hydatidosis-infected patients and 3 (3.3%) cases out of 90 control sera volunteers were detected to be false positive. This antigen did not show any false positive reaction with toxocariasis infected sera.

Various results were achieved from obtained peaks of chromatography gel filteration of the ELISA method adjacent to hydatid fluid crude antigens. Positive and negative predictive values of the ELISA test for various antigens were estimated and compared separately. The maximum positive indicative value belonged to antigen B (96.6%), and the minimum one belonged to P3G (70.6%). Maximum negative indicative value was related to P1G crude antigen (95.7%) and the minimum one was related to antigen B,P1G (88.2%).

Using the Dot - ELISA test, hydatid cyst crude antigen out of 60 sera infected with hydatidosis at 1:100- 1:200- 1:400 and 1:800 dilutions, 60 cases (100%) and out of 90 healthy control sera at mentioned dilutions, (67%), (40%), (33.3%) and (16.6%) cases respectively were detected to be false positive.

By using the Dot- ELISA method, purified antigen B out of 60 sera infected with hydatidosis at 1:100- 1:200 and 1:400 dilutions, 60 cases (100%) were positive, and out of 90 healthy control sera at mentioned dilutions (22.2%), (11.1%), (3.3%) and 0 cases respectively were detected to be false positive and the overall healthy control sera were negative.

To determine the sensitivity and specificity of Dot ELISA test, patients' sera were studied at 1:100, 1:200- 1:400- 1:800- 1:1600 and 1:3200 dilutions.

The sensitivity and specificity of Dot ELISA for each antigen were estimated and compared at various dilutions of sera and antigen B at dilution of 1:800, the sensitivity of 98.3 and specificity of 100% were detected to be appropriate dilutions to diagnose hydatid cyst.

Discussion

Serologic tests are among the best methods of diagnostic and epidemiologic study of hydatid cysts. Quick diagnosis of hydatid cysts is valuable.

A group of researchers; in order to estimate the diagnostic usage of post-surgery patients using a subclass of IgG to diagnose cases of hydatid cysts chose 28 samples of sera treated due to surgery and pharmaceutical treatment follow-up average for 5.6 years (3-12 years) . By using the ELISA method and crude antigen of hydatid cyst fluid, antibody modifications showed good correlation and radiological and clinical signs. It was observed that the concentration of antibodies IgG, IgG1 and IgG2 were greater than IgG3 and IgG4 comparatively and during antibody treatment, IgG2 showed the best correlation with patients' activities (21, 22).

Researchers, by using the ELISA method, antigens B and 5, and antibody IgG estimated the sensitivity and specificity of 97% and %95.7, respectively, and by using the same method and antigens but using IgM obtained the sensitivity of 37.5 % and specificity of 100% and using IgA the sensitivity of 45.5% and specificity of 98.9% (23, 24).

In the present study, although the sensitivity of crude antigen of hydatid cyst fluid using ELISA method was estimated to be 93.3% to diagnose patients infected with hydatid cyst, due to cross-reaction with sera infected with fascioliasis (46%), toxocariasis (15%), apparently healthy (5.7%), overall specificity (76.7%) and maximum experimental credits (85%) were obtained. Therefore, using crude antigens of hydatid cyst fluid may be useful only in the preliminary screening but they were not suitable for final and differential diagnosis of hydatidosis than the other parasitic and non-parasitic factors having cross-reaction with this disease.

Considering the above information and the need for a more specific serologic diagnosis of hydatidosis, after purification and separation of peaks, they were evaluated under the same condition, and diagnostic indications of each one were estimated, separately.

Among the evaluated different fractions, antigen B from the Oriol method 96.7% and P1, P2 of chromatography gel filtration 92.2 and 78.9 showed the maximum specific diagnosis of hydatidosis.

Considering diagnostic sensitivity, 88.3% antigen B, 95% P1 and, relatively low sensitivity 88.3% and 92.5% antigen B, 93.6% P1 were observed to have maximum diagnostic reliability among the different fractions.

By using the ELISA, cross and non-specific reactions of antigen B with sera infected with fascioliasis and healthy control were observed in 3.3% and P1 by using ELISA method with sera infected with fascioliasis and toxocariasis and healthy control was observed in 7.7%.

In various studies using fractions enriched with antigen B by ELISA method, sensitivity, and specificity were obtained. Rogan and his colleagues in 1991, in a study by Dot- ELISA and using antigen B fraction, reported an experimental sensitivity of roughly 98% and specificity of

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6.3% in which the reduced specificity of the experiment had been due to 28 sera infected with Cysticercus and 10 sera infected with alveolar cyst (25, 26).

Fortunately, due to the rare and limited number of alveolar cysts and the absence of any reports of infection with human Cysticercus in Iran, the probability of incidence of cross-reaction of two parasites was very rare.

Various studies, reported the sensitivity and specificity of the ELISA test using antigen B were 89% , and 85.7%, respectively and in the present study also significant cross-reaction of sera infected with alveolar cyst (50%) and Cysticercus (11%) caused the specificity decrease of antigen B with ELISA method (27, 28).

Another report showed a sensitivity of 63% and specificity of 100% by preparation and purification of a purer fraction of antigen B and by electroelution and evaluation by the ELISA method. Highly obtained specificity in this study apart from it can be due to the low number and type of control sera (overall , 12 cases infections with the other non-parasitic diseases including, schistosomiasis (n=8), filariasis and leishmaniasis (n=4)) , is mostly due to the quality and level of antigen B purity which has been prepared and purified by electroelution (17, 18).

However, the main point of mentioned reports and other similar studies were obtained to be similar to Shambesh et al. (1997) .Therefore, until now achieving highly great sensitivity and specificity simultaneously by antigen B using ELISA or any other hydatid cyst antigen has been impossible, and one of the main challenges and efforts such as these studies is to achieve this aim (29).

Therefore, published results of two different studies regarding evaluation of hydatid fluid antigen B, using the common methods of Dot ELISA and normal ELISA without providing new technology, the sensitivity and specificity of antigen B by Dot ELISA method has been reported 100%, 95%, respectively. In the other mentioned incidences of antigen B by ELISA method were reported to be 100%, and 97.5%, respectively (23, 24).

In various studies, the Dot-ELISA test was estimated by using purified antigens of Fasciola hepatica to diagnose the human hydatid serologically. In this study, sensitivity, specificity and indicative value were estimated to be 97.1%, 98.5%, 97.1% and 98.5% respectively (19, 20).

In the present study, the sensitivity of 98.3% and specificity of 100% of hydatid cyst were obtained by using Dot-ELISA at sera of 1:800 dilutions, in which the sensitivity was reduced at a higher dilution. Therefore, it was the suitable diagnostic method to find out patients that are cheap, available, simple and convenient to transport and applicable in most regions of the country. It indicates very high specificity and sensitivity.The testing period in the present study was about 2 hours, which is less than that of the maximum similar methods. Due to mentioned cases, the Dot- ELISA test can be a quick method to diagnose hydatid cysts, and it is worth in the preliminary stage of the test in the laboratory second stage as an epidemiologic test. ELISA compared the results of both stages and these results were almost the same whereas the specificity and sensitivity of Dot -ELISA is slightly higher than ELISA .The research which is done shown that antigen group B in the Dot- ELISA test has sensitivity and specificity. The highly obtained results of the present study also are to confirm the above-mentioned cases.

Conclusion

Hydatid cysts can exhibit a variety of symptoms. There might not have been a history of exposure to contaminated animals. For its diagnosis, a high level of clinical suspicion, together with a thorough history and clinical examination backed by laboratory testing, is needed. Dot-ELISA is a valid and potentially accurate method for diagnosing hydatid cyst infection. Present results showed that the Dot-ELISA was easy to perform, not expensive, safe, and with very good sensitivity and specificity

Conflict of Interests

The authors declare that they have no competing interests.

References

1.Vaisi-Raygani A, Mohammadi M, Jalali R, Salari N, Hosseinian-Far M. Prevalence of cystic echinococcosis in slaughtered livestock in Iran: SR/MAs. BMC Infect Dis. 2021;21(1):429.

2.Mahmoudi S, Mamishi S, Banar M, Pourakbari B, Keshavarz H. Epidemiology of echinococcosis in Iran: SR/MAs. BMC Infect Dis. 2019;19(1):929.

3.Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, World Health Organization. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Anim Health Res. 2001.

4.Al-Saeedi M, Khajeh E, Hoffmann K, Ghamarnejad O, Stojkovic M, Weber TF, et al. Standardized endocystectomy technique for surgical treatment of uncomplicated hepatic cystic echinococcosis. PLoS Negl Trop Dis. 2019;13(6):e0007516. 10.1371.

5.El Malki HO, Souadka A, Benkabbou A, Mohsine R, Ifrine L, Abouqal R, et al. Radical versus conservative surgical treatment of liver hydatid cysts. Br J Surg. 2014; 101(6):669–75.

6.Ramadan MM, El-Ameer AM, Shalash IR, Taki-El Deen FMA, Abdeen GS. Diagnosis of Echinococcosis (Hydatidosis) Using Dot-ELISA. Egypt J H Med. 2016;64:304- 310.

7.Bayram P, Nazmiye A, Nezahat A, Bruno G, Mikrobiyol B. Evaluation of the diagnostic value of the ELISA tests developed by using EgHF, Em2 and EmII/310 antigens in the serological diagnosis of alveolar echinococcosis. Mikrobiyol Bul. 2014;48(3):461-8.

8. Ingrid Reiter O, Beate G, Matthias F, Achim H, Peter K, Dennis T. Serological confirmatory testing of alveolar and cystic echinococcosis in clinical practice: results of a comparative study with commercialized and in-house assays. Clin Lab. 2009;55:41-8.

9. Grout A, Speakman E. In-flight transmission of foodborne disease: How can airlines improve. Travel Med Infect Dis. 2020;33:101558.

10.Yamano K, Goto A, Miyoshi M, Furuya K, Sawada Y, Sato N. Diagnosis of alveolar echinococcosis using immunoblotting with plural low molecular weight antigens. J Helminthol. 2009;83:57-61.

11.Deplazes P, Rinaldi L, Rojas CA, Torgerson PR, Harandi MF, Romig T, et al. Global distribution of alveolar and cystic echinococcosis. Adv Parasitol. 2017;95:315-493.

12.Mohammadzadeh T, Sako Y, Sadjjadi SM, Sarkari B, Ito A. Comparison of the usefulness of hydatid cyst fluid, native antigen B and recombinant antigen B8/1 for serological diagnosis of cystic echinococcosis. Trans R Soc Trop Med Hyg. 2012;106(6):371-5.

13. Abou-Elhakam HM, Farid AA, Mahana NA, Bauiomy IR. Dot-ELISA as a field test for hydatid diagnosis. AM J Egypt Soc Parasitol. 2016;46(2):441-452.

14. Zhang W, Li J, McManus D. Concepts in Immunology and Diagnosis of Hydatid Disease. Clin Microbiol Rev. 2003;16(1):18–36.

15. Golassa L, Abebe T, Hailu A. Evaluation of crude hydatid cyst fluid antigens for the serological diagnosis of hydatidosis in cattle. J Helminthol. 2011;85(1):100-8.

16. Zibaei M, Azargoon A, Ataie-Khorasgani M, Ghanadi K, Sadjjadi SM. The serological study of cystic echinococcosis and assessment of

[DOI: 10.47176/mjiri.37.87]

surgical cases during 5 years (2007-2011) in Khorram Abad, Iran. Niger J Clin Pract. 2013;16(2):221-5.
17.Tamarozzi F, Sako Y, Ito A, Piccoli L, Grisolia A, Itoh S, et al. Recombinant AgB8/1 ELISA test vs. commercially available IgG ELISA test in the diagnosis of cystic echinococcosis. Parasite Immunol. 2013;35(12):433-40.
18. Cisak E, Sroka J. Evaluation of reactivity to Echinococcus spp. among rural inhabitants in Poland. Acta Parasitol. 2015;60(3):525-9.
19. Nur Eris F, Akisu C, Aksoy U. Evaluation of Two ELISA and Two Indirect Hemagglutination Tests for Serodiagnosis of Pulmonary Hydatid Disease. Korean J Parasitol. 2009;47(4):427–429.
20. Lawn SD, Bligh J, Craig PS. Human cystic echinococcosis: evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. Am J Trop Med Hyg. 2004;70(3):329-35.
21. Fotoohi S, Hashemi GR, Borji TH. Serodiagnosis of human hydatidosis with an ELISA developed based on antigens derived from sheep hydatid cysts and comparison with a commercial human ELISA kit. Asian Pac J Trop Med. 2013;6(9):723-727.
22. Swarna S R, Parija SC. Dot-ELISA for evaluation of hydatid cyst wall, protoscoleces and hydatid cyst fluid antigens in the serodiagnosis of cystic echinococcosis. Rev Inst Med Trop Sao Paulo. 2008;50(4):233-6.
23. Darabi E, Motevaseli E, Mohebali M, Rokni MB, Khorramizadeh MR, Zahabiun F, et al. Evaluation of a novel Echinococcus granulosus recombinant fusion B-EpC1 antigen for the diagnosis of human cystic echinococcosis using indirect ELISA in comparison with a commercial diagnostic ELISA kit. Exp Parasitol. 2022;240:108339.
24. Zibaei M, Azargoon A, Khorasgani MA, Ghanadi K, Sadjjadi SM. The serological study of cystic echinococcosis and assessment of surgical cases during 5 years (2007-2011) in Khorram Abad. Iran Niger J Clin Pract. 2013;16(2):221-5,
25.Ghouraneh ME, Saberi M, Farhadineko S, Ahmadi K, Yousefi R, Mohammadzadeh T. Seroepidemiological study of hydatid cyst using AgB by ELISA in patients admitted to central laboratory of Baqiyatallah Hospital. Int J Enteric Pathog. 2019;7(1):15-8.
26. Sedaghat F, Sadjjadi SM, Hosseini SV, Kazemian S, Sarkari B. Evaluation of a Simple Dot-ELISA in Comparison with Countercurrent Immunoelectrophoresis for Diagnosis of Human Hydatidosis. Clin Lab. 2011;57:201-205
27.Chen X, Lu X, Feng X, Wen H. The production and comparative evaluation of native and recombinant antigens for the fast serodiagnosis of cystic echinococcosis with dot immunogold filtration assay. Parasite Immunol. 2015;37(1):10-5.
28.Eris FN, Akisu C, Aksoy U. Evaluation of two ELISA and two indirect hemagglutination tests for serodiagnosis of pulmonary hydatid disease. Korean J Parasitol. 2009;47 (4):427-9.
29. Govindasamy A, Raj P Bhattarai, John J. Liver cystic echinococcosis: a parasitic review. Ther Adv Infect Dis. 2023;10:1-12.

[DOI: 10.47176/mjiri.37.87]