




Sero-immunological Investigation of Cystic Echinococcosis: Comparison and Evaluation of Diagnostic Performances of Six Hydatid Cyst Antigens



Sara H. Draz¹, Ahmed Bayoumi², Raafat M. Shaapan^{1*} , Sherif Zidan²,
Nagwa I. Toaleb³ and Ghada Hadad²

¹Department of Zoonotic Diseases, Veterinary Research Institute, National Research Centre, 33 El-Bohouth St., Dokki, Giza, P.O. Box 12622, Egypt

²Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

³Department of Parasitology and Animal Diseases, Veterinary Research Institute, National Research Centre, 33 El-Bohouth St., Dokki, Giza, P.O. Box 12622, Egypt

Abstract

CYSTIC echinococcosis (CE) is a cyclozoonotic parasitic infection of worldwide distribution particularly in in developing countries and rural communities. Serological and immunological assays are useful for both for the initial diagnosis of infection in humans and animals as well as improving the quality of disease management. This study compares and assesses the efficacy of various hydatid cyst antigens for the accurate immunological diagnosis of CE in both humans and camels. Outer layer (Ol.C) germinal layer (Gl.C) fluid (Fl.C) and protoscolices (Ps.C) antigens prepared from camel hydatid cyst. In addition, fluid (Fl.H) and protoscolices (Ps.H) prepared from human hydatid cyst aspirate. All six-antigen types investigated as immunodiagnostic tools in human and camel hydatidosis using ELISA, SDS-PAGE and western blot assays. ELISA showed higher binding reactivity by protoscolices antigens (Ps.C and Ps.H) followed by fluid antigens (Fl.C and Fl.H), while outer and germinal layer antigens (Ol.C and Gl.C) give the lower activates. Ps.C antigen exhibiting higher sensitivity (100%), than Ps.H antigen (84.6 and 85.3%) against both positive camels and human sera. The sero-prevalence of Cystic echinococcosis was 59.78% and 43.65% in examined camel and human sera, respectively using the diagnostic Ps.C antigen. According to SDS-PAGE, all six hydatid cyst antigens demonstrated significant electrophoretic similarity, particularly in three bands of 42, 36, and 22 kDa, Using immunoblotting techniques, all separated antigens identified a sharing in two common bands with the same molecular weights of 42 a and 22 kDa. The obtained results concluded that camel and human hydatid cyst protoscolices antigens are introduced in this investigation as an effective diagnostic antigen and new immune-reactive fractions of 42 and 22 kDa for the diagnosis of hydatidosis in humans and camels.

Keywords: Hydatid cyst antigens, Camel, Human, ELISA, SDS, immune blotting.

Introduction

Cystic echinococcosis (CE) or hydatidosis, caused by the larval stage of *E. granulosus*, is an economically important global zoonotic infection that constitutes a threat to public health in many countries [1]. Hydatid cyst infection, also known as hydatidosis, is a cyclozoonotic parasite that is found

all over the world. The disease was just included in the WHO's strategic plan for combating neglected tropical diseases, and it poses a serious threat to public health and economic anxiety [2]. Cases involving humans and livestock animals, particularly camels, have been documented in Egypt. Because hydatidosis has no symptoms, it typically identified in necropsy [3]. It has been established that

*Corresponding author: Raafat M. Shaapan, E-mail: rshaapan2005@yahoo.com, Tel.: +201005280571 (Received 08/03/2024, accepted 26/03/2024)

DOI: 10.21608/EJVS.2024.275492.1899

©2025 National Information and Documentation Center (NIDOC)

serological immunological molecular assays are helpful not only in the initial diagnosis of infection in humans and animals but also in improving the quality of disease management and in monitoring patients following surgery or other treatments [4]. The socio-economic impact is considerably high since both man and livestock are involved as intermediate hosts [5]. The economic losses in animal production as lowered meat, milk and wool production and health hazard along with treatment costs of cystic echinococcosis in man are enormous [6]. Hydatid cysts can be spread in different organs of host such as liver, lung, heart, spleen and brain that may result in death [7]. Lung and hepatic hydatidosis injuries in livestock animals cause economic loss due to the condemnation of tissues [8].

Camels (*Camelus dromedaries*), are mostly found in hot, desert regions of the world, such as the Middle East, Africa, and India. With a population of more than 37 million, camels are a significant source of milk and meat in many marginal and desert regions of the world [9]. Due to the growing human demand for meat, especially in Egypt and other African nations, the absence of biosecurity laws and biosafety in many areas, the expansion of camel herds in wildlife with nondomestic species, and other factors, the potential for camels to act as disease vectors are extremely concerning [10]. Hydatid cyst affects 1–220 people per 100,000 depending on the region. The disease is endemic in Mediterranean and Middle Eastern countries; eastern European countries; and in eastern Africa, Australia, China, New Zealand, and Argentina [11]. Egypt is a country located in North Africa that is home to about 110 million people. About 57% of Egyptians reside in rural areas and 43% in cities that have seen urbanization [12]. Egypt has an exceptional biodiversity, a pleasant temperature, and a large agricultural sector. These elements create the perfect setting for the spread of many diseases, including parasites such as the endemic *Echinococcus granulosus* parasite in Egypt [13].

Based on their characteristics, hydatid cysts are divided into three groups: sterile hydatid cysts, which are fluid-filled but lack protoscoleces; fertile hydatid cysts, which have protoscoleces and/or daughter cysts; and calcified hydatid cysts, which have a toughened wall and no protoscoleces [14]. The usual structure of hydatid cysts looked to have an outer layer called the cuticular membrane and an inner layer called the germinal layer, along with cellular infiltration and a fibrous tissue capsule found in the liver and lung tissue affected by the infection [15]. Early detection of CE using serology may offer chances for more successful chemotherapy, early treatment, and post-treatment follow-up [16]. There is no medical need to perform a biopsy just for genotyping when the diagnosis of CE is established by non-invasive imaging, especially in light of the

possibility of subsequent cyst formation linked to invasive diagnostic techniques. Imaging, serology, and frequently even histopathology, however, cannot provide a definitive diagnosis of the causative species or intraspecific entity [17]. The using of crude hydatid cyst antigens in serologica diagnostic tests are not full adequate, thus, the purification of hydatid cyst antigens is obligatory to exclude the other cross reactive proteins [18].

Several investigations have carried out to enhance the effectiveness of serological examinations and enable the identification of low concentrations of antibodies against cystic echinococcosis. The assessment of different hydatid cyst antigens is contingent upon several factors, such as antigen stability, purity, source, and laboratory protocols [19]. Numerous methods, including the enzyme-linked immunosorbent test (ELISA) and the enzyme-linked immune-electro-transfer blot (EITB), are used for this type of diagnosis in both humans and various animals [20]. Since ELISA is often less expensive, effective, has good sensitivity and specificity, is easy to use, and requires fewer qualified workers, it is considered the preferable test for identifying many parasitic infections in domestic animals and people [21]. However, the specificity of the antigens diminished by its cross-reaction with proteins overexpressed in other diseases, many methods, including SDS-PAGE electrophoresis and immunoblotting, used to extract proteins from hydatid cyst protoscoleces. This has improved the characterization of hydatid cyst antigenic proteins and produced highly sensitive and specific results for the diagnosis and prognosis of hydatidosis [22]. Camels in Egypt and other countries reported to have echinococcosis and hydatidosis in a variety of ecological zones, and the prevalence of infection were found to be an epizootiological trait linked to infection rates in the intermediate host reservoir animals [23]. Therefore, this study investigated to compare and evaluate the performance of various hydatid cyst antigens for the accurate immunological diagnosis of cystic echinococcosis in both humans and camels.

Material and Methods

Animal samples

Camel tissue samples

The hydatid cysts will be collected from livers and lungs of camels slaughtered at Kom-Hamada abattoir, Beheira Governorate, Egypt. To maintain normal physiological tissue conditions, the cysts were first stored in iceboxes before promptly moved to a laboratory using phosphate buffer saline (PBS). The cyst regarded as sterile since it lacked protoscolices and had pus, calcified, or deteriorated inside of it. In the investigation, only viable cysts with protoscolices were used [24].

Camel blood samples

280 camel blood samples were obtained at the time of slaughtering, 12 positive samples were collected from infected slaughtered camels with lung cysts and 18 blood samples from healthy camels free from cysts (negative samples) as proved by veterinary inspection after slaughter and 250 random camel blood samples were collected. Sera were prepared, divided into small aliquots and stored at -20 °C for serological and immunological analysis.

Human samples

Hydated cyst fluid

Human hydatid cyst fluid aspirated under aseptic instances during therapeutic procedures. After centrifuging hydatid fluid for ten minutes at 400xg, the sediment collected and looked at under a light microscope. When a cyst is surgically removed and pathologically determined to be a hydatid cyst, the aspirated cyst fluid can also be obtained from that site [25].

Human blood samples

A total of 285 blood samples were drawn from patients presenting with fever, stomach pain, and digestive disorders in private clinics and outpatient clinics. Where 15 of these samples obtained from patients who confirmed infected with hydatidosis by sonographer and IHAT (positive control), 30 blood samples from healthy individuals of no hydatidosis and IHAT negative (negative control) and other 240 are randomly collected samples.

Camel hydatid cysts antigens

Hydatid cyst fluid antigen (Fl.C)

Aseptic aspiration of hydatid cyst fluid performed in a sterile tube from fertile cysts. The mixture was centrifuged at 6000xg for 45 minutes, filtered, and dialyzed with saline buffered with phosphate. Hydatid cyst fluid finally lyophilized and kept at 4° C [26].

Protoscolices antigen (Ps.C)

The fertile camel hydatid cysts were punctured aseptically to extract the protoscolices. The resulting hydatid cyst fluid then centrifuged, and the protoscolices were separated and placed in a sterile tube. The protoscolices were centrifuged at 6000 xg for 30 minutes while on ice in a 150 w ultrasonic disintegrator in PBS until no intact protoscolices were visible under a microscope. The supernatant was then aliquoted and kept at -20°C [27].

Outer and Germinal layer Hydatid cyst wall antigens (Ol.C & Gl.C)

The cysts' fluid extracted, and the walls were sliced and opened using forceps for carefully remove the exterior and inner (germinal) tissues. To verify

that there are no remnants of the protoscolices, the separated outer and inner layer extracts microscopically inspected. After that, the two layer preparations were sonicated for 15 minutes on ice using a 150W ultrasonic disintegrator and maintained at -20°C [28].

Human hydatid cyst antigens

Hydatid cyst fluid Antigen (Fl.H)

The aspirated fluid of viable hepatic cyst, which collected during surgery by sterile puncture, was centrifuged at 6000 xg for 15 minutes, and the resulting supernatant was then filtered using a Millipore filter, lyophilized, and kept at 4° C and used antigen preparation [29].

Protoscolices Antigen (Ps.H)

Protoscolices were detected under a microscope in aspirated human hydatid cyst fluid, and the sedimentation of protoscolices was centrifuged. The sediment of protoscolices sonicated in PBS, ultra centrifuged and kept at -20° C [30].

Protein content determination

The protein content of each of the six prepared antigens produced from hydatid cysts derived from camel and human was measured [31].

Serological investigation

Enzyme Linked Immunosorbant Assay (ELISA)

ELISA was adopted to assess the potency of protoscolices and both outer and germinal layer antigens. Check board titration was used to establish the ideal concentrations of antigen, serum, and conjugate before test procedures were executed [32].

Antigens characterization (SDS-PAGE)

To characterize the antigens on polyacrylamide gels under reducing circumstances, 10% SDS-PAGE was used. Before loading the antigens onto the gel, each produced antigen mixed separately with a buffer containing 2-mercaptoethanol. Silver stain was used to color the fixed gel in 50% methanol [33]. The pre-stained high and low molecular weight samples used and electrophoresed on the same gel after the relative molecular weights of the bands were calculated [34].

Immunoblot

Naturally infected camel sera used to incubate the nitrocellulose membrane, then a 1:200 diluted ELISA used to evaluate the membrane. The horseradish peroxidase-conjugate was diluted 1:10,000. The second membrane was analyzed using diluted 1:200 ELISA after treated with spontaneously infected human sera. A 1:2000 dilution of horseradish peroxidase-conjugated anti-human IgG was utilized. The 4-chloro-1-naphthol solution (Sigma) was added to the two membranes to clean them [35].

Results

The potency of bound antigens with hydatid cyst antibodies

ELISA adopted to prove the binding activities of antibodies cystic echinococcosis in both naturally infected human and camel sera with cystic echinococcosis towards the fall prepared six hydatid cysts antigens. The profiles binding reactivity in

naturally infected human and camel sera showed that the higher activates by protoscolices antigens (Ps.C and Ps.H) followed by fluid and antigens (Fl.C and Fl.H), while outer and germinal layer antigens (Ol.C and Gl.C) give the lower binding activates (**Figure 1 A & B**). Moreover, the protoscolices camel antigens (Ps.C) is the most potent of binding capabilities in both naturally infected human and camel sera than the other all antigens (**Figure 2**).

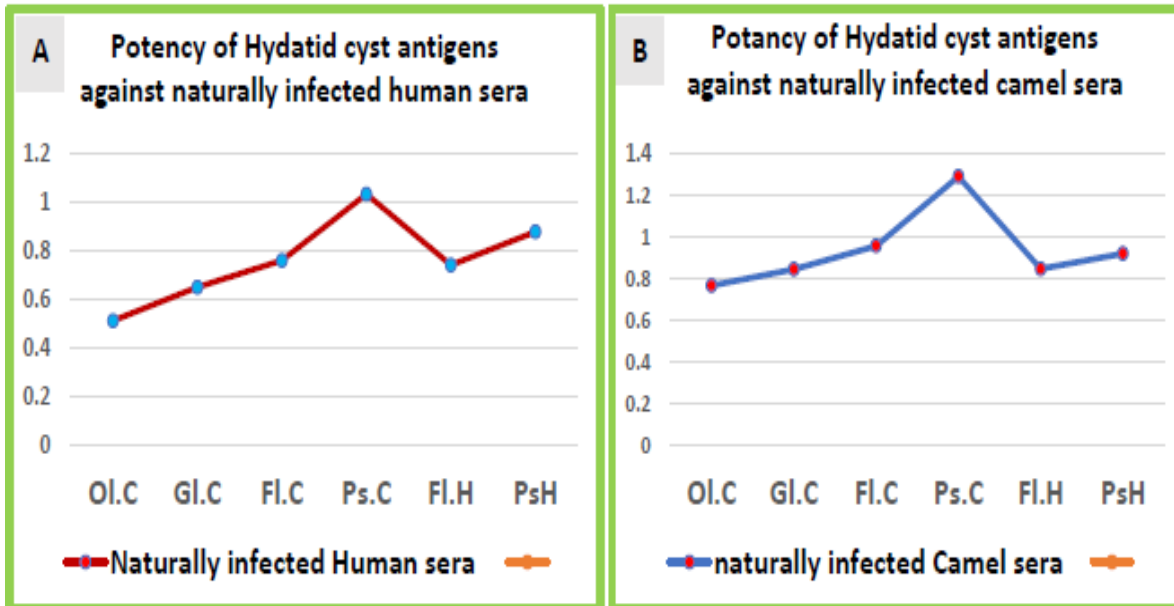


Fig. 1. Potency of hydatid cyst antigens against both naturally infected human (A) and camel (B) sera

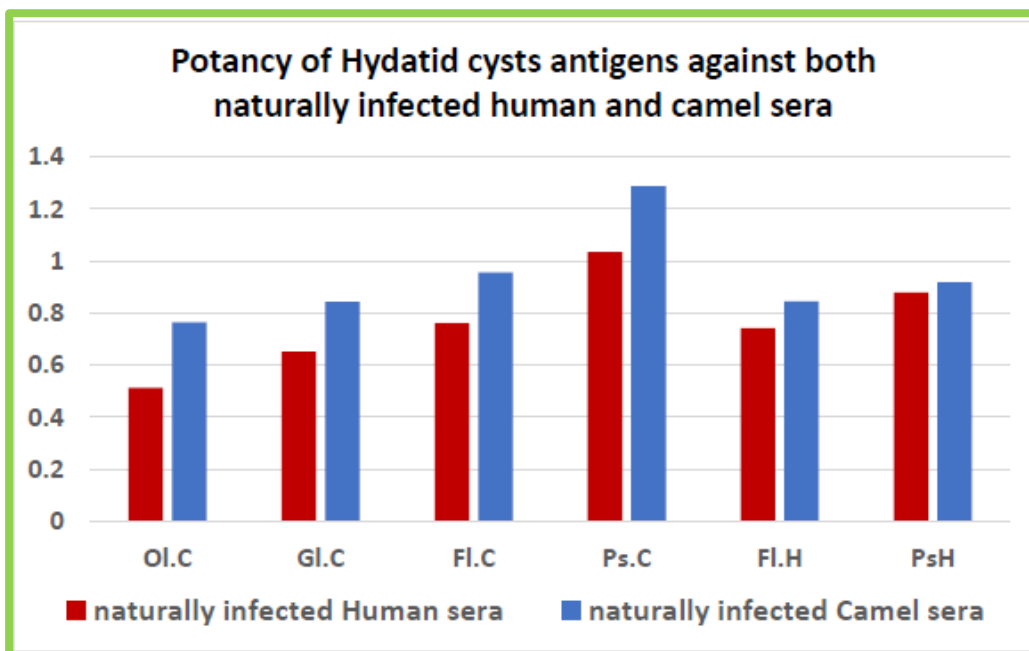


Fig. 2. Protoscolices camel (Ps.C) antigens is the most potent binding reactivity in both naturally infected human and camel sera than the other all antigens

Sensitivity and specificity of protoscolices antigens

Both Ps.C and Ps.H antigens applied for the diagnosis of hydatidosis among naturally infected (positive) and healthy (negative) of both camel and human sera using ELISA. All the positive camels and human serum samples reacted positively with the camel protoscolices (Ps.C) antigen exhibited 100%

sensitivity, while the specificity of Ps.C antigen recorded 95.65% and 88.23% values with healthy (negative) camel and human sera respectively, at cut off value 0.315 (Figure 3). On the other hand human protoscolices (Ps.H) antigen demonstrated 84.6% and 85.36% sensitivity, while the specificity was 76.47% and 73.91% against the positive and negative camels and human serum samples, respectively at cut off value 0.257 (Figure 4).

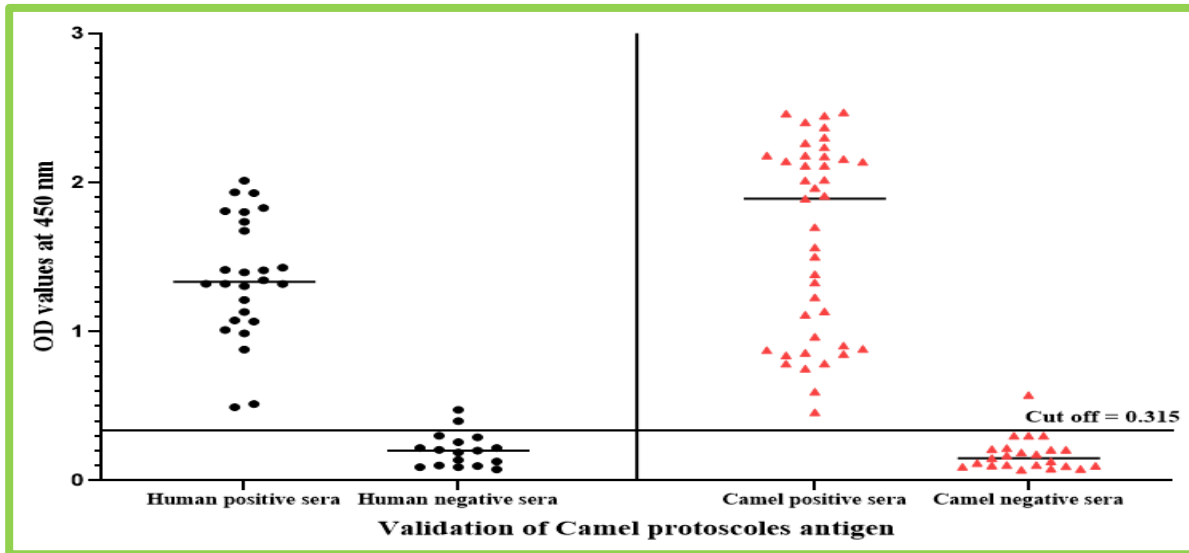


Fig. 3. Diagnostic activity of Ps.C antigen for hydatidosis in naturally infected and negative human and camel sera

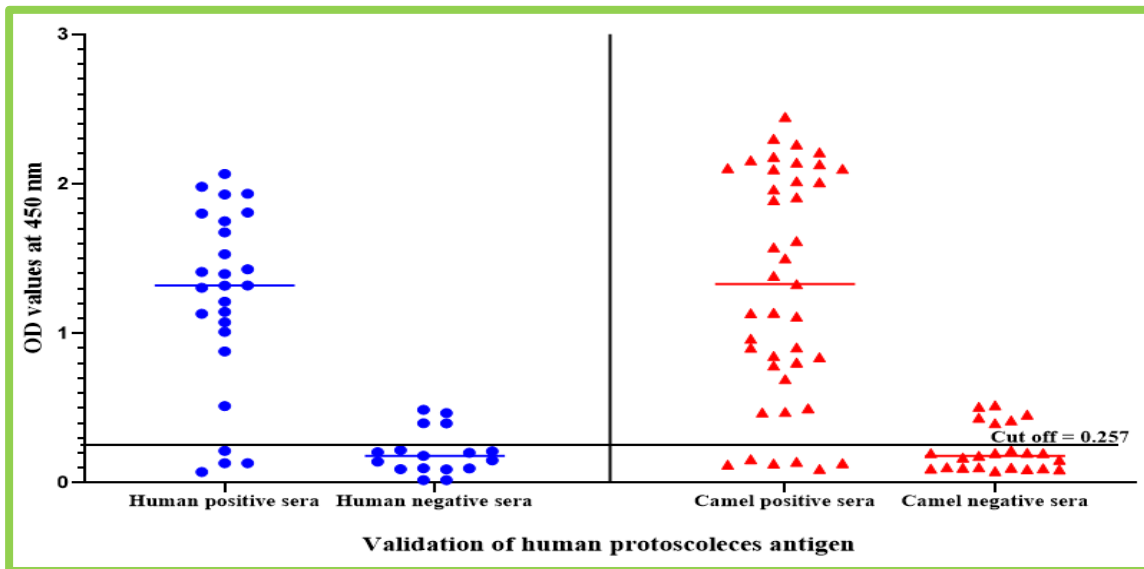


Fig. 4. Diagnostic activity of Ps.H antigen for hydatidosis in naturally infected and negative human and camel sera

The sero-prevalence of hydatidosis in camels and human

The antibody IgG detection of cystic echinococcosis antibodies in random camel and human sera adopted by ELISA using Ps.C antigen prepared from camel hydatid cyst, for coating the two ELISA plates. The potency of the Ps.C antigen in the diagnosis of cystic echinococcosis, recorded

that the percentage of detected antibodies was 43.65% in these collected random human sera at cut off value 0.582 (**Figure 5 A**), whereas, 59.78% of antibodies to cystic echinococcosis was detected in collected random camel sera at cut off value 0.626 (**Figure 5 B**).

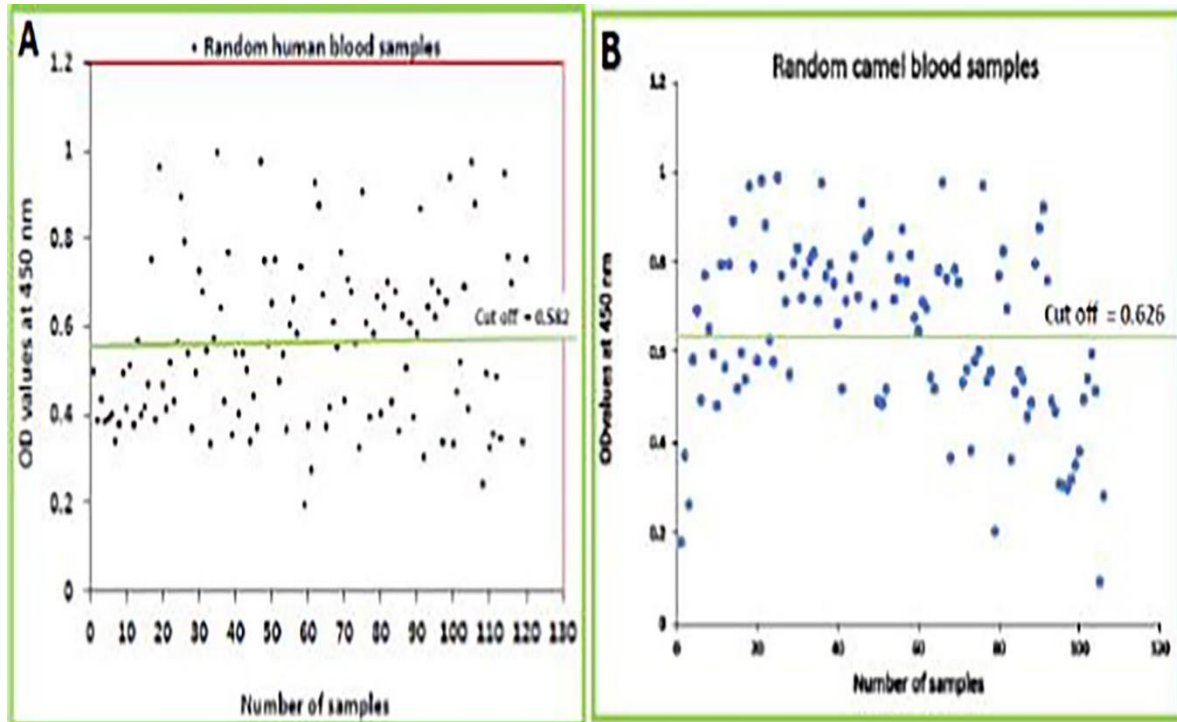


Fig. 5. Diagnostic potency of Ps.C antigen in diagnosis of hydatidosis in human (A) and camel (B)

Electrophoretic profile of the hydatid cyst antigens

Electrophoresis process for the prepared six hydatid cyst antigens at 10% SDS-PAGE under reducing condition. The camel hydatid cyst antigens; the outer layer (Ol.C), revealed seven bands (95, 66, 55, 42, 36, 32 and 22) kDa. The protoscolices (Ps.C) antigen detected 6 band (207, 58, 42, 36, 22 and 22) kDa. The fluid (Fl.C) antigen detected seven bands (58, 42, 40, 36, 26, 22 and 12) kDa. The germinal layer (Gl.C) show six bands (66, 55, 42, 36, 22 and 19) kDa. Whereas the human hydatid cyst, protoscolices and fluid (Ps.C & Fl.H) antigen detected only three band (42, 36 and 22) kDa. It was demonstrated extensive electrophoretic similarity between the six antigens especially in three bands of 42, 36 and 22 kDa (**Figure 6**).

Immunogenic reactive components of antigens by Immunoblotting

Immunogenic reactive components in camel hydatid cyst antigens; four camel hydatid cyst antigens; outer layer (Ol.C), protoscolices (Ps.C), fluid (Fl.C) and inner layer (Gl.C) and the two human hydatid cyst antigens, protoscolices (PsH) and fluid (Fl.H) were recognized by naturally infected camel sera. There were give sharing in two common bands at the same the molecular weights 42 kDa and 22 kDa (**Figure 7 A**).

Furthermore, naturally, infected human sera identified the same common immunogenic bands in six antigens at 42 kDa and 22 kDa, beside other immunogenic band 36 kDa. While, the outer (Ol.C) and inner germinal layer hydatid cyst camel antigens (GLC) shared at 55 kDa (**Figure 7 B**).

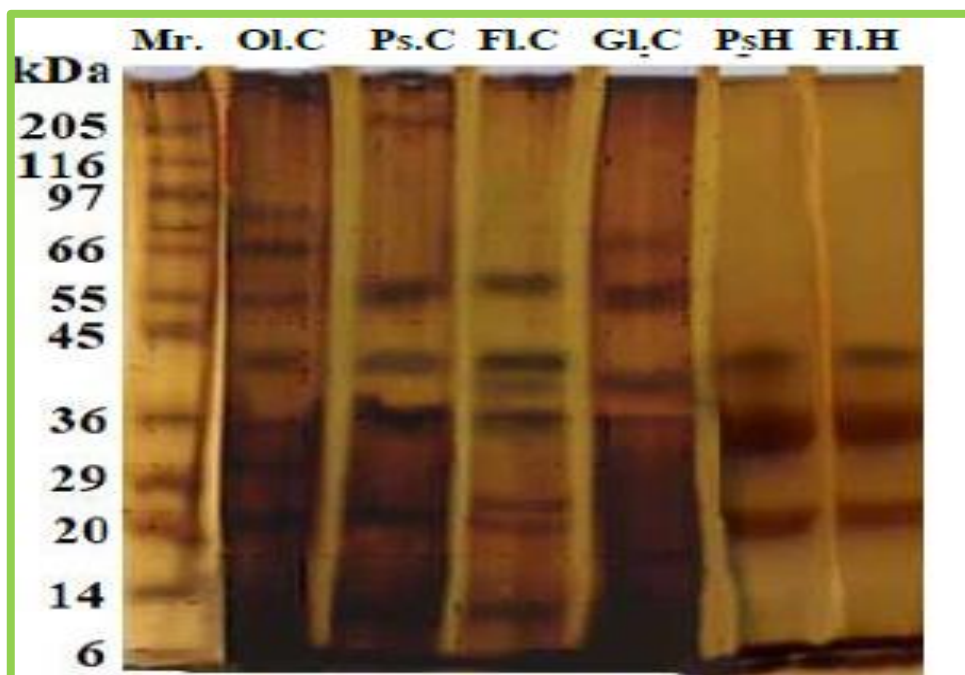


Fig. 6. Comparative SDS-PAGE of four camel hydatid cyst antigens (Ol.C, Ps.C, Fl.C and Gl.C), two human hydatid cyst antigens (Ps.H and Fl.H), and molecular weight standards in kDa; (Mr)

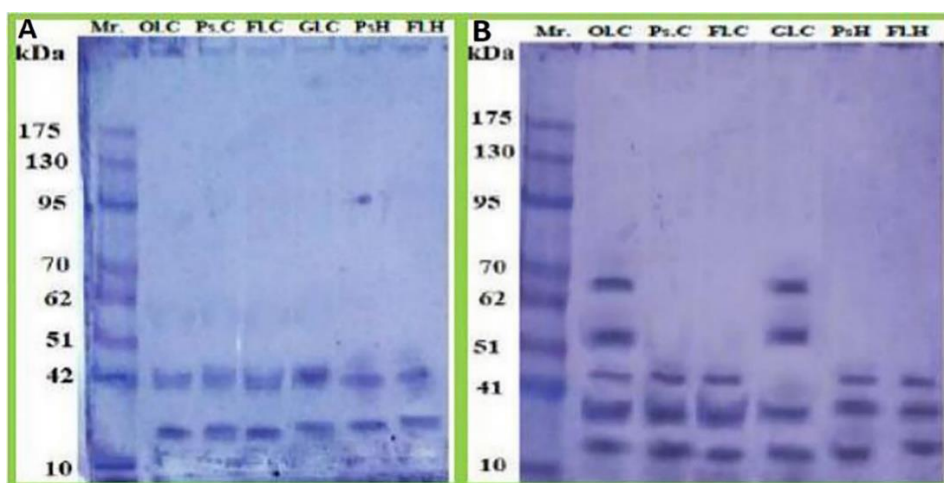


Fig. 7. Immunoreactive bands against 4 camel hydatid cyst antigens (Ol.C, Ps.C, Fl.C and Gl.C), 2 human hydatid cyst antigens (Ps.H and Fl.H) and molecular weight standards in kDa (Mr) recognized by naturally infected camel sera (A) and naturally infected human sera (B)

Discussion

The development of alternative diagnostic techniques, such as antibody detection using ELISA, urgently needed and serological assays, on the other hand, could provide a powerful pre-slaughter diagnostic screening tool for cystic echinococcosis in animals. This could reduce the spread of *E. granulosus* on farms and in the environment, important for the prevention of human infections [36, 37]. In the present study, the profiles binding reactivity in naturally infected human and camel sera

in our finding showed that the higher activates by protoscolices antigens (Ps.C and Ps.H) followed by fluid antigens (Fl.C and Fl.H), while outer and germinal layer antigens (Ol.C and Gl.C) give the lower activates. These findings corroborate those from earlier ELISA studies that looked at instances of hydatidosis in humans and camels and demonstrated that the protoscolex was the most useful diagnostic antigen. Furthermore, finding a single, distinct antigen among the protoscolices antigens will require more research in order to

improve their diagnostic effectiveness. The antigens isolated from the protoscolices of hydatid cysts are a viable option for the serological diagnosis of human cystic echinococcosis (CE) [38]. Conversely, research focused on producing two refined antigen (hydatid cyst fluid and protoscoleces) from sheep showed that the hydatid cyst fluid antigen was more effective than the protoscoleces antigen [39]. However, relatively little research has been directed toward the development of immunodiagnosis techniques for *Echinococcus granulosus* infection in human and domesticated animals using a different of antigenic preparations [40]. The variation in antigenic potency and effectiveness amongst the different prepared hydatid cyst antigens could be attributed mostly to elevated high protein concentrations or to altered purification techniques utilized during antigen synthesis [41].

ELISA regarded as the preferred test for diagnosing many parasitic diseases in domestic animals and humans since it is generally less expensive, effective, has good sensitivity and specificity and needs fewer skilled workers [42]. In this work ELISA was adopted to evaluate the sensitivity and specificity of the both Ps.C and Ps.H antigens in the diagnosis of hydatidosis among naturally infected and healthy both camel and human sera. The Ps.C antigen exhibited 100% sensitivity, while recorded a specificity of 95.65% and 88.23%. On the other hand, Ps.H antigen demonstrated 84.6% and 85.36% sensitivity, but 76.47% and 73.91% specificity. Our results somewhat similar reported studies revealed sensitivity for the crude protoscolices antigens in the ELISA system, were ranged from 90 to 100% and specificity from 75% to 78% [43, 44]. Our findings, however, were greater in sensitivity than of two other investigations conducted by other researchers that employed protoscolices for the primary serodiagnosis of CE [45, 46]. The majority of serological techniques employed in diagnosis have their own issues and constraints, including restricted availability, inconsistent sensitivity and specificity, availability of laboratory equipment, and origin and kind of antigens utilized. Consequently, in the clinical context, the outcomes of serological studies are contingent upon various aspects, including antigen quality, composition, concentration, and stability, afflicted organ, number of cysts (individual or multiple), and individual immunological response variability [47].

Despite this, developing countries continue to have a high prevalence of CE, especially in rural regions, cystic echinococcosis (CE) is diagnosed, the quality of illness management and treatment can be significantly improved [5]. This study found that the sero-prevalence of cystic echinococcosis by ELISA using Ps.C antigen was 43.65% in random human sera and 59.78% in random camel sera. Comparable ELISA values for CE prevalence in randomly

selected camel and human serum samples were 54.79% and 61.32%, respectively [43]. Studies conducted among communities have shown that in endemic areas, the frequency of human CE ranges from 1% to 10% [48]. Remarkably, the slaughterhouses in different governorates in Northern and Southern Egypt accounted more of 25% of the camels that were slaughtered [49]. In human a serological survey of CE, 3% to 5% and 6% using IHA were seropositive for hydatidosis, while the seroprevalance was higher (64%) using ELISA [50]. Compared to people living in cities, inhabitants of rural areas were more vulnerable to contracting CE. Among the occupational categories, the increased incidence rate of CE in farmers and related populations was linked to Egyptian agriculture and dog care [51]. The discrepancy in the prevalence of CE in human and slaughtered camels was due to the type of serological tests used, the effectiveness and purification of the antigens used, and seasonal factors [52].

Using SDS-PAGE in the current investigation, all six hydatid cyst antigens demonstrated significant electrophoretic similarity, particularly in three bands of 42, 36, and 22 kDa. Significant electrophoretic similarity was found between the five purified hydatid cyst antigens (FLh, Psh, FLc, Psc, and GLc) in a somewhat comparable SDS-PAGE analysis, particularly in three common shared reactive bands (52, 41, and 22 kDa) [43]. A variety of methods, including SDS-PAGE electrophoresis have been used to extract proteins from hydatid cyst protoscoleces and express them to better characterize hydatid cyst antigen proteins for diagnostic and prognostic purposes [53]. The electrophoresis evaluation of different hydatid cystic prepared antigens (layers, fluid and protoscoleces) as substitute antigen sources utilizing immunological diagnostic techniques is an essential method for sero-diagnosis of cystic echinococcosis infection [39]. In this study, western immunoblotting techniques investigated that the all purified six antigens recognized a sharing in two common bands at the same molecular weights of 42 kDa and 22 kDa against the naturally infected camel and human sera. Similarly, bands detected by crude antigens made from protoscoleces, cyst walls, and hydatid cyst fluid after reactions against sera from human infected with hydatid cysts [54]. In addition, likewise, shared immune-reactive bands of 52 and 41 kDa found against both naturally infected human and camel sera by some pure fluid and protoscoleces hydatid cyst antigens [43]. The main problem with the results of the western immunoblotting tests in diagnosis of hydatidosis and this variation may be related to the sample size of the study or batch of the antigen, which has been used in the given tests [18].

Conclusions

In this investigation, the protoscolices camel (Ps.C) and human (Ps.H) antigens are the most potent of binding capabilities and can be successfully used to diagnose hydatidosis in both camel and human sera using the ELISA test, which is useful to control infection and reduce human transmission. Moreover, this sero-immunological study introduces camel hydatid cyst protoscoleces (Ps.C) as effective diagnostic antigens and new immune-reactive fractions of 42 and 22 kDa for the diagnosis of human and camel cystic echinococcosis.

Acknowledgment

The authors would like to acknowledge with thanks all the staff of the Zoonotic Disease Department, National Research Centre, Egypt who provided help and support during the research.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical considerations

All experimental methods carried out in compliance with Ethical protocol no.18/234, the institutional rules established by the Animal Research Committee of the National Research Centre

References

- Cui, Q., Zhang, Q. and Hu, Z. Modeling and analysis of Cystic Echinococcosis epidemic model with health education. *AIMS Mathematics*, **9**(2), 3592-3612 (2024). DOI: 10.3934/math.2024176
- Gessese, A.T. Review on epidemiology and public health significance of hydatidosis. *Veterinary Medicine International*, **2020**, 8859116 (2020). DOI: 10.1155%2F2020%2F8859116
- Toaleb, N.I. and Shaapan, R.M. Zoonotic Protozoan Parasites Infecting Camels, Diagnosis and Control—A Review. *Egyptian Journal of Veterinary Sciences*, **55**(4), 1131-1142 (2024). DOI: 10.21608/ejvs.2023.251609.1686
- Shaapan, R.M., Toaleb, N.I. and Abdel-Rahman, E.H. Detection of *Toxoplasma gondii*-specific immunoglobulin (IgG) antibodies in meat juice of beef. *Iraqi Journal of Veterinary* **35**, 319–324 (2021). DOI:10.33899/ijvs.2020.126829.1390
- Mathivathani, C., Ajaykumar, V.J. and Bora, C. Epidemiology and Public Health Significance of Hydatidosis: A Review. *Current Journal of Applied Science and Technology*, **42**(25), 19-26 (2023). DOI: 10.9734/cjast/2023/v42i254182
- Ahmed, M.A., Ahmed, C. and Mengistu, A. A Study on Prevalence and Economic Significance of Bovine Hydatidosis in Haramaya Muncipal Abattior. *Journal of veterinary Medicine and Animal Science*. **7**(1), 1135 (2024) DOI: study-on-prevalence-.pdf
- Elaadli, H., El Adly, H., M Shaapan, R.M. and Bessat, M., An Uncommon Primary Splenic Hydatid Cyst in Human: A Case Report Study. *The Egyptian Journal of Hospital Medicine*, **89**(1), 5493-5497 (2022). DOI: 10.21608/ejhm.2022.264847
- Abo-Aziza, F.A., Oda, S.S., Aboelsoued, D., Farag, T.K. and Almuzaini, A.M. Variabilities of hydatidosis in domestic animals slaughtered at Cairo and Giza abattoirs, Egypt. *Veterinary World*, **12**(20), 998 (2019). DOI: 10.14202%2Fvetworld.2019.998-1007
- Fereig, R.M., Mazeed, A.M., El Tawab, A.A.A., El-Diasty, M., Elsayed, A., Shaapan, R.M., Abdelbaset, A.E., Frey, C.F., Alawfi, B.S., Altwaim, S.A. and Alharbi, A.S. Exposure to Brucella Species, Coxiella burnetii, and Trichinella Species in Recently Imported Camels from Sudan to Egypt: Possible Threats to Animal and Human Health. *Pathogens*, **13**(2), 179 (2024). DOI: 10.3390/pathogens13020179
- Alanazi, A.D., Abdullah, S., Helps, C., Wall, R., Puschendorf, R., ALHarbi, S.A., Abdel-Shafy, S. and Shaapan, R.M. Tick-Borne Pathogens in Ticks and Blood Samples Collected from Camels in Riyadh Province, Saudi Arabia. *Int. J. Zoo. Res.*, **14**(1), 30-36 (2018). DOI: 10.3923/IJZR.2018.30.36
- Petropoulos, A.S. and Chatzoulis, G.A. Echinococcus granulosus in childhood: A retrospective study of 187 cases and newer data. *Clinical Pediatrics*, **58**(8), 864-888 (2019). DOI: 10.1177/0009922819847032
- World Population Review. Egypt population. Population of Cities in Egypt 2024. (2024). <https://worldpopulationreview.com/countries/cities/egypt>
- Khatib, S., Sobeh, M., Faraloni, C. and Bouissane, L. Tanacetum species: Bridging empirical knowledge, phytochemistry, nutritional value, health benefits and clinical evidence. *Frontiers in Pharmacology*, **14**,1169629 (2023). DOI: 10.3389/fphar.2023.1169629
- Gareh, A., Saleh, A.A., Moustafa, S.M., Tahoun, A., Baty, R.S., Khalifa, R.M., Dyab, A.K., Yones, D.A., Arafa, M.I., Abdelaziz, A.R. and El-Gohary, F.A. Epidemiological, morphometric, and molecular investigation of cystic echinococcosis in camel and cattle from upper Egypt: current status and zoonotic implications. *Frontiers in Veterinary Science*, **8**, 750640 (2021). DOI: 10.3389%2Ffvets.2021.750640
- Shoulah, S.A., Gaballa, M.M., Marawan, M.A., Saqr, S.A., Abdelhady, A., Alzahrani, H.A., Wakid, M.H., Al-Jabr, O.A. and Selim, A. Pathological Findings and Oxidative Stress Status Associated with Hydatidosis in Dromedary Camels. *Veterinary Sciences*, **10**(2),74(2023). DOI: 10.3390%2Fvetsci10020074
- El-Aswad, B.E.W., Abd El-Atty, A.F., El-Melegy, M.A., Shaapan, R.M., Atia, A.F., El Kaddy, N.A. and Abd El-Ghaffar, M.M. Roles of Inducible Nitric Oxide Synthase and Interleukin-17 in Mice Experimentally Infected with *Trypanosoma evansi*. *International Journal Zoological Research*, **14** (1), 21-29 (2018). DOI: 10.3923/IJZR.2018.21.29

17. Siles-Lucas, M., Uchiumi, L. and Tamarozzi, F. 'No cyst, no echinococcosis': a scoping review update on the diagnosis of cystic echinococcosis after the issue of the WHO-IWGE Expert Consensus and current perspectives. *Current Opinion in Infectious Diseases*, **36**(5), 333-340 (2023). DOI: 10.1097/QCO.0000000000000941
18. Hassanain, M.A., Shaapan, R.M. and Khalil, F.A.M. Sero-epidemiological value of some hydatid cyst antigen in diagnosis of human cystic echinococcosis. *Journal of Parasitic Diseases* **40**(1), 52-56 (2016). DOI: 10.1007/s12639-014-0443-5
19. Hoge, M.O., Ciomaga, B.F., Muntean, M.M., Muntean, A.A., Popa, M.I. and Popa, G.L. (Cystic Echinococcosis in the Early 2020s: A Review. *Tropical Medicine and Infectious Disease*, **9**(2), 36 (2024). DOI: -infectiousdiseases/toc/2023/10000
20. Darwish, D.A., Masoud, H.M., Helmy M.S., Abbas, W.T., Shaapan, R.M. Toaleb N.I. and Ibrahim, M.A. Isolation, characterization, and ELISA applications of alkaline phosphatase and acetylcholinesterase from *Moniezia expansa*. *Iraqi Journal of Veterinary Science* **38**(1), 215-223 (2024). DOI: 10.33899/ijvs.2023.142183.3161
21. Shaapan, R.M., El-Nawawi, F.A. and Tawfik, M.A. Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. *Veterinary Parasitology*, **153**(3-4), 359-362 (2008). DOI: 10.1016/j.vetpar.2008.02.016
22. Sedaghat, B., Hajjarian, H., Sadjjadi, F.S., Heidari, S. and Sadjjadi, S.M. Proteomic characterization of hydatid cyst fluid: two-dimensional electrophoresis (2-DE) setup through optimizing protein extraction. *BMC Research Notes*, **14**(1), 1-6 (2021). DOI: 10.1186/s13104-020-05433-3
23. El-Salem, R.M., Khan, A. and Younis, E.Z. Cystic echinococcosis in slaughtered animals in Libya: A Review. *Journal of Pure & Applied Sciences*, **20**(1), 167-171 (2021). DOI: 10.51984/jopas.v20i1.1239
24. Khan, S.N., Ali, R., Khan, S., Norin, S., Rooman, M., Akbar, N.U., Khan, T.A., Haleem, S., Khan, M.A. and Ali, I. Cystic echinococcosis: an emerging zoonosis in southern regions of Khyber Pakhtunkhwa, Pakistan. *BMC Veterinary Research*, **17**(1), 1-11. (2021). DOI: 10.1186/s12917-021-02830-z
25. Bothale, K., Kolhe, H., Mahore, S. and Wilkinson, A. Diagnosis of primary hydatid cyst of thigh by fine needle aspiration cytology. *Indian Journal of Medical Microbiology*, **33**(1), 151 (2015). DOI: 10.4103/0255-0857.148426
26. Toaleb, N.I., Helmy, M.S., El Shanawany, E.E. and Abdel-Rahman, E.H. A simple and efficient purification method of native immunoreactive antigen for diagnosis of camel hydatidosis. *Veterinary World*, **13**(1), 141 (2020). DOI: 10.14202/vetworld.2020.141-146
27. Wang, H., Li, J., Guo, B., Zhao, L., Zhang, Z., McManus, D.P., Wen, H. and Zhang, W. In vitro culture of *Echinococcus multilocularis* producing protoscoleces and mouse infection with the cultured vesicles. *Parasites & Vectors*, **9**, 1-6 (2016). DOI: 10.1186/s13071-016-1687-y
28. Taherkhani, H. and Rogan, M.T. Preparation of semi-purified laminated layer of hydatid cyst for diagnosis of infection. *Archive Razi Institute*, **52**, 61-74 (2001). DOI: 83520015207.pdf
29. Gadea, I., Ayala, G., Diago, M.T., Cunat, A. and de Lomas, J.G. Immunological diagnosis of human cystic echinococcosis: utility of discriminant analysis applied to the enzyme-linked immunoelectrotransfer blot. *Clinical. Diagnostic. Laboratory Immunology*, **6**(4), 504-508 (1999). DOI: 10.1128/cdli.6.4.504-508.1999
30. Rafiei, A. and Craig, P. The Immunodiagnostic Potential of Protoscolex Antigens in Human Cystic Echinococcosis and the Possible Influence of Parasite Strain. *Ann. Trop. Med. Parasitol.*, **96**(4), 383-389 (2002). DOI: 10.1179/000349802125001195.
31. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275 (1951). DOI: 10.1016/S0021-9258(19)52451-6
32. Hassanain, N.A., Shaapan, R. M., Hassanain, M. A. and Zaky, S. Comparison Between Insecticidal Activity of Lantana camara Extract and its Synthesized Nanoparticles Against Anopheline mosquitoes. *Pakistan Journal of Biological Sciences*, **22**(7), 327-334 (2019). DOI: 10.3923/PJBS.2019.327.334
33. Toaleb, N.I., Shaapan, R.M., Hassan, S.E. and El Moghazy, F.M. High Diagnostic Efficiency of Affinity Isolated Fraction in Camel and Cattle Toxoplasmosis. *World Journal of Medical Science*, **8**(1), 61-66 (2013). DOI: 10.5829/idosi.wjms.2013.8.1.72161
34. Abouelsoued, D.M., Shaapan, R.M., Elkhateeb, R.M.M., Elnattat, W.S. and Hammam, A.M. Therapeutic efficacy of ginger (*Zingiber officinale*), ginseng (*Panax ginseng*) and sage (*Salvia officinalis*) against *Cryptosporidium parvum* in experimentally infected mice. *Egyptian Journal of Veterinary Sciences*, **51**(2), 241-251 (2020). DOI: 10.21608/EJVS.2020.24183.1152.
35. Hassan, S.E., Shaapan, R.M., Ghazy, A.A. and Abdel-Rahman, E.H. Evaluation of purified horse derived antigen in the diagnosis of human toxoplasmosis. *Glob. Vet.*, **9**, 630-634 (2012). DOI: gv/GV9(5)12/20.pdf
36. Elfadaly, H.A., Hassanain, M.A., Shaapan, R.M., Barakat, A.M. and Toaleb, N.I. Serological and hormonal assays of murine materno-fetal *Toxoplasma gondii* infection with emphasis on virulent strains. *World J. Med. Sci.*, **7**(4), 248-254 (2012). DOI: wjms/7(4)12/7.pdf
37. Wen, H., Vuitton, L., Tuxun, T., Li, J., Vuitton, D.A., Zhang, W. and McManus, D.P. Echinococcosis: advances in the 21st

- century. *Clinical Microbiology Reviews*, **32**(2), 1110-1128 (2019). DOI: 10.1128/CMR.00075-18
38. Shahabinejad, P., Shahriarirad, R., Omidian, M., Ghorbani, F., Barazesh, A. and Sarkari, B. Diagnostic performance of Echinococcus granulosus protoscolices antigens in the serodiagnosis of human cystic echinococcosis. *Journal of Immunoassay and Immunochemistry*, **41**(5), 833-840 (2020). DOI: 10.1179/000349802125001195
39. Siddartha, P.V., Babu, A.J., Rao, T.M., Rayulu, V.C. and Swetha, C.S. A comparative evaluation of four different immunoassays in the diagnosis of cystic echinococcosis using a crude and purified hydatid cyst fluid antigen. *Acta Parasitologica*, **67**(4), 1667-1679 (2022). DOI:10.1007/s11686-022-00614-5
40. Peruzzu, A., Mastrandrea, S., Fancellu, A., Bonelli, P., Muehlethaler, K., Masala, G. and Santucci, C. Comparison and evaluation of analytic and diagnostic performances of four commercial kits for the detection of antibodies against *Echinococcus granulosus* and *multilocularis* in human sera. *Comparative Immunology, Microbiology and Infectious Diseases*, **86**, 101816 (2022). DOI: 10.1016/j.cimid.2022.101816
41. Maleki, F., Akhlaghi, L. and Tabatabaie, F. Evaluation of Hydatid Cyst Antigen for Serological Diagnosis. *Medical Journal of the Islamic Republic of Iran*, **37**, 87 (2023). DOI: 10.47176%2Fmjiri.37.87
42. Shaapan, R.M., Toaleb, N.I. and Abdel-Rahman, E.H. Significance of a common 65 kDa antigen in the experimental fasciolosis and toxoplasmosis. *Journal of Parasitic Diseases*, **39**(3), 550-556 (2015). DOI: 10.1007/s1263901303942
43. Hassanain, M.A., Toaleb, N.I., Shaapan, R.M., Hassanain, N.A., Maher, A. and Yousif, A.B. Immunological detection of human and camel cystic echinococcosis using different antigens of hydatid cyst fluid, protoscolices, and germinal layers. *Veterinary World*, **14**(1), 270 (2021). DOI: veterinaryworld.org/Vol.14.2021/35.pdf
44. Simsek, S. and Koroglu, E. Evaluation of enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) for immunodiagnosis of hydatid diseases in sheep. *Acta Tropica* **92**(1), 17-24 (2004). DOI: 10.1016/j.actatropica.2004.04.006
45. Dyab, A.K., Marghany, M.E., Othman, R.A., Ahmed, M.A. and Abd-Ella, O.H. Hydatidosis of camels and sheep slaughtered in Aswan Governorate, southern Egypt. *Российский паразитологический журнал*, **12**(3), 33-41 (2018). DOI: 10.31016/1998-8435-2018-12-3-33-41
46. Ahmed, A.B., Ras, R., Mahmoud, A.F., El-Ghazaly, E., Widmer, G., Dahshan, H. and Elsohaby, I. Prevalence and bacterial isolation from hydatid cysts in dromedary camels (*Camelus dromedarius*) slaughtered at Sharkia abattoirs, Egypt. *Journal of Parasitic Diseases*, **45**, 236-243 (2021). DOI: 10.1007/s12639-020-01300-x
47. Alsakee, H.M. Diagnostic Efficiency of Heat Shocked Protoscolices Extract Antigens for Human Cystic Echinococcosis by ELISA. *Iraqi Journal of Science*, **37**, 65-74 (2023). DOI: 161053a19122b004
48. Acosta-Jamett, G., Hernández, F.A., Castro, N., Tamarozzi, F., Uchiumi, L., Salvitti, J.C., Cueva, M. and Casulli, A. Prevalence rate and risk factors of human cystic echinococcosis: A cross-sectional, community-based, abdominal ultrasound study in rural and urban north-central Chile. *PLOS Neglected Tropical Diseases*, **16**(3), p.e0010280 (2022). DOI: 10.1371/journal.pntd.0010280
49. Shawish, R.R., AbouLaila, M.R., Elkhtam, A.O., El-Bahrawy, A., Omar, M.A., Hadad, G.A., Meshhal, H.F. and Elbayoumi, Z.H. Prevalence, molecular characterization, and economic impact of hydatid cysts in the slaughtered animals in abattoirs of Minoufyia governorate, Egypt. *Journal of Advanced Veterinary Research*, **13**, 857-864 (2023). DOI: index.php/AVR/article/view/1316
50. El-Ghareeb, A.S., Waked, N.M. and Al-Feky, H.M. Clinical and parasitological studies on pulmonary and hepatic hydatid cysts in hospitalized children and adults. *Journal of the Egyptian Society of Parasitology*, **46**(1), 9-18 (2016). DOI: 10.21608/jesp.2016.88925
51. Abdelbaset, A.E., Yagi, K., Nonaka, N. and Nakao, R. Cystic echinococcosis in humans and animals in Egypt: An epidemiological overview. *Current Research in Parasitology & Vector-Borne Diseases*, **1**, 100061 (2021). DOI: 10.1016/j.crpvbd.2021.100061
52. Yakubu, R.A., Nock, I.H., Ndams, I.S., Luka, S.A., Yaro, C.A., Alkazmi, L. and Batiha, G.E.S. Detection of Echinococcus granulosus sensu lato cysts and seroprevalence of cystic echinococcosis in cattle and camels in Maiduguri Abattoir. *Journal of Parasitic Diseases*, **46**(3), 876-888 (2022). DOI: 10.1007%2Fs12639-022-01508-z
53. Siracusano, A., Teggi, A. and Ortona, E. Human cystic echinococcosis: old problems and new perspectives. *Interdisciplinary Perspectives on Infectious Diseases*, **2009**, 474368 (2009). DOI: 10.1155/2009/474368
54. Niazi, M., Saki, M., Sepahvand, M., Jahanbakhsh, S., Khatami, M. and Beyranvand, M. In vitro and ex vivo scolicidal effects of *Olea europaea* L. to inactivate the protoscolices during hydatid cyst surgery. *Annals of Medicine and Surgery*, **42**, 7-10 (2019). DOI: 10.1016/j.amsu.2019.04.006

التقص المناعي المصلي لداء المشوكات الكيسي: مقارنة وتقييم الأداء التشخيصي لسنة مستضدات من الأكياس العدرية

سارة حمادة دراز¹ ، أحمد محمد بيومي² ، رأفت محمد شعبان^{1*} ، شريف عبد الله زيدان² ، نجوى إبراهيم تعيلب³ وغادة عبد المنصف حداد²

^{1*} قسم الأمراض المشتركة - معهد البحوث البيطرية - المركز القومي للبحوث- الجيزة- مصر.

² قسم الصحة والأمراض المشتركة- كلية طب بيطري - جامعة مدينة السادات - مصر.

³ قسم الطفيليات وامراض الحيوان- معهد البحوث البيطرية- المركز القومي للبحوث- الجيزة- مصر.

داء المشوكات الكيسي هو عدوى طفيلية مشتركة بين الإنسان والحيوان منتشرة في جميع أنحاء العالم وخاصة في البلدان النامية والمجتمعات الريفية. وتعتبر الاختبارات المصلية والمناعية مفيدة لكل من التشخيص الأولي للعدوى لدى البشر والحيوانات وتحسين جودة مكافحة المرض. وتهدف هذه الدراسة بمقارنة وتقييم فعالية مستضدات (أنتيجينات) الأكياس العدرية (المائية) المختلفة للتشخيص المناعي الدقيق لمرض المشوكات الكيسي في كل من البشر والإبل. فقد تم تحضير أنتيجينات الطبقة الخارجية (O.I.C) والطبقة الجرثومية (G.I.C) والسائل (F.I.C) و البذور الأولية (P.S.C) من أجزاء الأكياس المائية المعزولة من الجمال بالإضافة إلى ذلك، تم تحضير أنتيجينات السائل (F.I.H) والبذور الأولية (P.S.H) من مستخلص الأكياس المائية المعزولة من البشر. وتم فحص جميع أنواع المستضدات الستة كأدوات تشخيصية مناعية لمرض الأكياس المائية (المشوكات الكيسي) في البشر والإبل باستخدام اختبار الأليزا والفصل الكهربائي وفحوصات الطبع المناعي. أظهرت نتائج تقنية الأليزا أعلى تفاعل ارتباط بواسطة أنتيجينات البذور الأولية (P.S.C & P.S.H) و تليها أنتيجينات السوائل (F.I.C & F.I.H) ، في حين كان تفاعل الارتباط اقل بواسطة أنتيجينات الطبقتان الخارجية والطبقة الداخلية (O.I.C & G.I.C) . وقد أظهر الأنتيجين P.S.C أعلى حساسية (100%)، من الأنتيجين P.S.H (84.6) و (85.3%) ضد كل من امصال الجمال والأمصال البشرية وامصال الإبل التي تم فحصها، على التوالي باستخدام المستضد الكيسي 59.78% و 43.65% في الأمصال البشرية وامصال الإبل التي تم فحصها، على التوالي باستخدام المستضد التشخيصي P.S.C. وبواسطة تقنية الفصل الكهربائي SDS-PAGE، أظهرت جميع الأنتيجينات الستة المحضرة من الأكياس المائية تشابهاً كبيراً في التمييز الكهربائي، خاصة في ثلاثة نطاقات هي 42 و 36 و 22 كيلو دالتون، وباستخدام تقنيات الطبع المناعي أظهرت جميع الأنتيجينات المنفصلة مشاركة في نطاقين مشتركين لهما نفس الأوزان الجزيئية البالغة 42 و 22 كيلو دالتون. لذلك في هذا البحث تم استنتاج تقديم أنتيجينات البذور الأولية من الأكياس العدرية في الإبل والإنسان كمستضد تشخيصي فعال وكذلك تم فصل أجزاء جديدة مناعية متفاعلة تبلغ 42 و 22 كيلو دالتون للتشخيص الدقيق لمرض الأكياس المائية في البشر والإبل.

الكلمات الدالة: أنتيجينات الكيس المائي، الإبل، الإنسان، الأليزا، الفصل الكهربائي، الطبع المناعي