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Original Article

Sero- evaluation of cattle cystic echinococcosis using different antigens of hydatid cyst fluid, protoscolices, germinal and laminated layers

Mona Mohammed I. Abdel Rahman[1]*, Abdallah Fikry A. Mahmoud[2], Abd El-Salam E. Hafez[2], Rania Helmy M. Shata[2], Rasha M. El Bayomi[2].

[1]Department of Parasitology,

[2]Department of Food Hygiene, Safety and Technology Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt

***Corresponding author:**
mona_111_para@yahoo.com
01124983310

Abstract:

Cystic echinococcosis (CE), caused by *Echinococcus* granulosus, affects both humans and livestock. Final hosts (dogs) are typically subclinical, while intermediate hosts show symptoms based on cyst location and infection severity. CE is mainly detected in abattoirs via post-mortem (PM) examination, making diagnosis challenging and increasing transmission risk. The current study aimed to evaluate the serodiagnosis and statistical significance of using different prepared antigens from cysts against PM method. 170 blood samples were collected from cattle and the separated serum samples were prepared for detection of optical density values by indirect ELISA against different hydatid cysts antigens. The antigens were isolated from various cystic components. Crude hydatid cyst fluid antigen (HCF Ag) was prepared by aspirating fluid from fertile cyst separated from infected organs, centrifuged and aliquoted, laminated layer antigen (LL Ag) obtained by aspirating fluid, separating the laminated layer and homogenized, germinal layer antigen (GL Ag) was isolated through dissection, protoscolices antigen (Pscs Ag) collected, washed with PBS, and all isolates sonicated, and stored at -20°C. Protein concentration was measured using modified Lowry method. The obtained values were statically analyzed. PM prevalence was 11.76%. While ELISA showed 13.5%, with higher prevalence in the liver (7.06%) than lungs (2.35%). Significant differences were noted between antigens, with GL, HCF, and Pscs Ags showing higher values than LL Ag. ELISA diagnostic accuracy was 98.24 % for LL, GL, and Pscs Ags, and 60% for HCF Ag. Our study suggests that GL, HCF, and Pscs Ags were effective and sensitive immunodiagnostic tools for screening hydatosis.

Keywords:

Cystic Echinococcosis, Cysts, *Echinococcus* Infection, Hydatid, Hydatidosis

INTRODUCTION

Post mortem inspection of meat is a good indicator for detection of parasitic infections among the slaughtered animals in abattoirs. As a result, it assists in monitoring parasites in the national herd through providing feedback information to the veterinary service to control disease and to protect the public from zoonotic hazards **(Birhanu et al. 2015)**.

Cystic echinococcosis (CE) is worldwide zoonotic, parasitic disease caused by the larval stage of *Echinococcus granulosus* **(Rogan and Craig 1997)**. During the slaughtering process, if hydatid cysts in infected animal organs rupture, they could release protoscolices and other cyst contents into the environment. Secondary echinococcosis may occur as a result of the

liberated protoscolices becoming new cysts. The risk of direct contact with cyst fluid was high for individuals handling these infected organs. Accidental ingestion of the parasite's eggs or leakage of protoscolices from ruptured cysts during surgical removal can lead to human infection. This risk is heightened if proper hygiene practices, such as wearing protective gloves and thoroughly washing hands, are not followed. To reduce these risks, it is essential to use appropriate meat inspection methods, ensure safe handling and disposal of infected offal, and observe rigorous hygiene practices throughout and after slaughtering **(Da Silva 2024; Fallah et al. 2014)**.It is highly registered in temperate zoned countries like north and east Africa **(Grosso et al. 2012)**.CE induce huge economic losses which resulted from decrease in gained body weights, milk and meat productivity, hide quality, and costs for disposal of condemned organs, animal treatment and control measures **(Budke et al. 2006 and Wahlers et al. 2012).**

Echinococcus species have two-host life cycle where the adult worms inhabit in small intestine of dogs as a main host. They produce the fertilized eggs which pass in faeces and contaminate food and water of intermediate hosts like sheep, cow, pig, camels, goat, buffalo, as well as human. Then, they hatch in I.H. intestine and releasing oncospheres which penetrate the gut and reach liver or lung via portal or lymph circulation. Liver and lung considered the main predilection site for hydatid cysts development, but they may reach other organs like the kidney, pancreas, central nervous system, or marrow cavity of long bones. The cyst consisted of an inner germinal layer and outer laminated layer, surrounded by fibrous adventitia as a local-host defense mechanism reaction, filled with fluid. After several months, the germinal layer produce broad capsules and protoscolices.

The infection is usually a symptomatic in definitive host **(Zajac et al. 2021)**.While, in intermediate hosts, the symptoms were varied according to the cyst location, involved infected organs, and degree of infection. In liver can produce hepatic insufficiency, digestive disturbances, and ascites, in lungs can induce dyspnea, in brain; cerebral symptoms might appear (paralysis, blindness, etc.). PM examination in abattoirs considered the main tool for infection diagnosis which made the diagnosis difficult and increases the risk of zoonotic transmission. Human considered an accidental intermediate host where they acquired infection during direct close contact with dogs or via contaminated hands with adult worm eggs **(Paniker 2007).** However, the clinical symptoms were developed later after the cyst growth to bigger sizes. The induced symptoms were mainly resulted from pressure atrophy of enlarged cysts on the involved or surrounding organs, or from complications of cyst fluid leakage. The leakage may induce an allergic (Type I) reaction and anaphylactic shock due to hypersensitivity against *Echinococcus* antigen component **(Kern et al. 2017 and Gottstein et al. 2017)**. Also, infected patients with uncomplicated liver or lung cysts may suffer from abdominal or chest pain and respiratory distress. The infection with CE in humans diagnosed mainly by ultrasound technique.

There is no chemical specific treatment of hydatid cysts in domestic animals. In human, the surgical operation for removal of HC offers the best mode of treatment, but their recurrence after surgery is common **(Paniker 2007)**. Mebendazole and Albendazole therapies can be administered after operations to prevent metastatic infections from spillage of cyst contents **(Porter and Kaplan 2011).**

The aim of this study is to determine (a) the prevalence rate of hydatid cyst through post mortem examination VS. indirect ElISA test, (b) to evaluate the efficiency of different prepared antigens by ElISA, (c) to detect the significance of the obtained results from PM

and ElISA test, and finally (d) to determine test accuracy by different prepared antigens.

MATERIAL AND METHODS

Samples collection and preparation of sera:

Hydatid cysts, during postmortem examination of cattle slaughtered at Dakahlia abattoir in Egypt, liver and lung hydatid cysts were collected to prepare different antigens.

Serum samples, total of 170 cattle blood samples were collected during slaughtering at Dakahlia abattoir. The post mortem documented 20 positive samples (cattle either with lung cyst or liver cyst separately or both of them) and 150 negative samples (free from cysts), by a visual inspection. By centrifugation at 1000 rpm for 10 min, the serum samples were obtained, collected and stored at -20^{0}c.

Antigens preparation:

Laminated layer crude antigen (LL Ag), the hydatid cyst fluid was aspirated with a syringe and examined microscopically for protoscolices. The laminated layer was separated carefully from the inner germinal layer with sterile forceps, homogenized, sonicated, centrifuged and the supernatant stored at -20^{0}c (**Taherkhani and Rogan, 2000).**

Hydatid cyst germinal layer antigen (GL Ag)**,** after aspiration of hydatid fluid aseptically, the cysts were dissected with sterile scissor to scrap and isolate the inner germinal layer from the outer laminated layer. Then continue freezing-thawing cycles, sonication, centrifugation, and keeping at -20^{0}c until use **(Hassanain et al. 2021)**.

Crude hydatid cyst fluid antigen (HCF Ag), the hydatid fluid was aspirated from lung and liver with sterile needle. The obtained fluid was centrifuged at 1000 rpm for 30 min to remove any protoscolices or debris and then the supernatant was aliquoted and kept at -20^{0}c until use **(Fotoohi et al. 2013 and El-Kattan et al. 2020).**

Protoscolices antigen (Pscs Ag), the protoscolices were collected from fertile bovine hydtid cysts by aseptic puncture. They were washed with phosphate-buffered saline (PBS; pH 7.2), followed by freezing-thawing cycle for three times, sonicated, and left overnight at 4^{0}c. The sonicated mixture was centrifuged at 1000 rpm for 30 min to collect the supernatant and stored at -20^{0}c **(Fotoohi et al. 2013).**

The total protein concentrations for the prepared four Ags were determined by Lowry's method **(Lowry et al. 1951).**

ELISA procedures:

Polystyrene flat-bottom 96-well microplates were coated with 5 µg of each of the four antigens derived from cattle in a coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6). The plates were incubated overnight at room temperature (RT) to allow for antigen binding. The following day, the plates were washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20 to remove any unbound antigens. After washing, the plates were blocked with 200 µl of bovine serum albumin (BSA) solution to prevent non-specific binding. The blocking step was done by incubating the plates at RT for 2 hours. After blocking, the plates were washed again with PBS-Tween solution. Next, 100 µl of diluted serum samples (1:200 dilution, determined via checkerboard titration to be the optimal dilution) were added to each well. The plates were incubated at RT for 30 minutes to allow for antigen-antibody binding. After incubation, the plates were washed again to remove any unbound serum. Then, 100 µl of protein A peroxidase conjugate from Staphylococcus aureus (HPR) diluted to 1:2000 (Sigma Chemicals, St. Louis, Missouri, USA) was added to each well and incubated at RT for 30 minutes. After washing, 100 µl of o-phenylene diamine (OPD) substrate solution (Sigma) was added to each well, and the plates were incubated for 30 minutes to allow for color development. The reaction was stopped and the optical density (OD) was measured at 490 nm using an ELISA reader to assess the antigen-antibody interaction **(Golassa et al. 2011).**

Statistical methods:

The data were analyzed using SPSS version 25 (Armonk, NY: IBM Corp), and NCSS version12 (LLC. Kaysville, Utah, USA). The data were expressed as frequency and percentage. Chi-square test was run to test the association between breed and infection and between type of test (ELISA and PM) and infection. Cochran's Q test followed by Minimum Required Absolute Difference test was run to assess the differences in proportion of infection in three organs (liver, lung, both). The level of significance was set to be < 0.05. One-way ANOVA was run to test differences among antigens. The optimal cut-off points between positive and negative samples was estimated using the Youden index, maximizing the difference between true positive rate (sensitivity) and false positive rate (1 - specificity). Thereby, the maximum of sensitivity and specificity is achieved. The degree of agreement between the methods after categorization according to optimal cut-off was measured using the intra-class correlation. Receiver operating characteristic (ROC curve) analysis was used to find out the overall predictivity of parameter, and to find out sensitivity and specificity at this cut-off value; Sensitivity = (true +ve)/ [(true +ve) + (false –ve)], Specificity = (true –ve) / [(true –ve) + (false +ve)] (Golassa et al. 2011). Z-test and Youden index used for comparing AUC (which indicate diagnostic accuracy) of two diagnostic tests.

Ethics statement:

This study was approved ethically by ZU-IACUC committee, Zagazig University, Egypt with number ZU-IACUC/2/F/286/2022

RESULTS

PM examination:

By PM examination, the total prevalence rate reached 11.76%. It was higher in liver (7.06%) than lung (2.35%) (Table 1&2), and Fig.1.

There was a non-significant relationship between involved breeds and prevalence of hydatid cyst in infected cattle p > 0.05. However, there was a statistically significant difference between involved organs and prevalence rates. A pairwise post-hoc Minimum Required Absolute Difference test showed a highly significant difference for lung vs. liver (p < 0.01) and liver vs. both (liver &lung, p < 0.01), while lung vs. both showed non-significant difference (P> 0.05) Table 1&2.

ELISA assay:

The obtained prevalence rate by ELISA reached 13.5%. The ELISA & PM examination methods, as indicated in Table 3 and Fig. 2, had a highly significant impact on the positivity percentages (p < 0.0001) for LL, Gl, and Pscs Ags, and (0.000006) for HCF Ags. Table 3 showed the disparity between HCF Ag and the other Ags, with HCF showing a 23% true positive percentage while the other Ags showed 86.96%. Furthermore, HCF reported a false negative percentage of 77%, while other Ags reported 13.04%. According to Table 4 and Fig. 3, there was a significant distinction between different antigens (p < 0.01), as GL, HCF, and Pscs Ags had higher OD values than LL Ag.

Accuracy of indirect ELISA by different HC antigens:

From (Tables 5, 6& 7), Fig. 4 & 5, we can determine the diagnostic parameters of the four used Ags in our study. AUC of LL, GL Ag, and Pscs Ags reached 100%, which indicated an excellent (98.24%)diagnostic accuracy of ELISA test, while AUC of HCF Ag reached 0.56 which that indicated a bad (60%) diagnostic accuracy. The differences between using GL & HCF Ag, HCF& Pscs Ag, and HCF & LL Ag= (0.442) showed statistical significance p < 0.05. In spite of using, LL&GL Ags, LL & Pscs Ags, and GL & Pscs Ag = (0.0) (P > 0.05) did not show any significant differences. The sensitivity reached 100% for all used Ags. The specificity reached 98% for LL, GL & Pscs Ags while, it reached the lowest record (55.8%) in case of using HCF Ag. From the previously obtained results, LL, GL and Pscs Ags considered the best in performance in comparison with HCF Ag. From the obtained ELISA values, we can determine the degree

of agreement which appeared moderate to good between the following antigen pairs: LL& GL Ags, LL & Pscs Ags, and Gl & Pscs Ags. While, it appeared poor among pairs of antigens (LL & HCF Ags, GL&HCF Ags, and HCF & Pscs Ags) (Table 8).

Table 1: Hydatid cyst prevalence rate among breed and organs categories.

		Total number	No. of infected	Infection %	
breed	native	37	4	10.81	
	crossbreed	133	16	12.03	$> 0.05^{NS}$
Overall number		170	20	11.76	
organ	lung	170	4	2.35	
	liver	170	12	7.06	0.02*#
	Both liver and lung	170	4	2.35	

NS: non-significant difference p >0.05; * p < 0.05 denotes statistical significant; # Cochran's Q test p-value.

Table 2: Proportion of infection according to involved organs using minimum required absolute difference test

	Absolute difference
Lung vs. liver	4.71*
Lung vs. liver &lung together	0^{NS}
Liver vs. liver &lung together	4.71*

NS: non-significant difference p >0.05; * p < 0.05 denotes statistical significant.

Table 3: The proportions of positive and negative responses to various antigens when utilizing ELISA and PM examination methods.

GL, LL, and Pscs Ags				
		ELISA		
		Positive	Negative	
PM	Positive	20/23 (86.96%)	0/147 (0.0%)	144.87(<0.0001)
	Negative	3/23 (13.04%)	147/147 (100%)	
		HCF Ag ELISA		
		Positive	Negative	
PM	Positive	20/87 (23%)	0/83 (0.0%)	18.88(0.000006)
	Negative	67 /87 (77%)	83/83 (100%)	

GL: Germinal layer, HCF: Hydatid cyst fluid, LL: laminated layer, Pscs: Protoscolices.

Table 4: The mean and standard deviation (SE) for the ELISA tests on serum samples that were subjected to various antigens prepared from hydatid cysts.

	Mean± SE
LL Ag	0.24±0.006[b]
GL Ag	0.27 ± 0.008[a]
HCF Ag	0.27±0.008[a]
Pscs Ag	0.25±0.005[ab]

ab Means with different superscript are statistically different p< 0.05, GL: Germinal layer, HCF: Hydatid cyst fluid, LL: laminated layer, Pscs: Protoscolices.

Table 5: AUC (area under curve) of LL, GL, HCF, and Pscs with the Youden index.

	AUC	Sig.	Youden index
LL Ag	1 (0.98 - 1)	< 0.0001	> 0.318
GL Ag	1 (0.98 - 1)	< 0.0001	> 0.4
HCF Ag	0.56 (0.48 -0.63)	> 0.05	> 0.23
Pscs Ag	1 (0.98 - 1)	< 0.0001	> 0.321

AUC: area under curve, GL: Germinal layer, HCF: Hydatid cyst fluid, LL: laminated layer, Pscs: Protoscolices.

Table 6: Comparisons of the difference between AUC of antigen pairs.

Variable	Difference between areas (AUROC)	SE	95% CI	z statistic	P value
LL Ag vs. Gl Ag	0	0	0	-	> 0.05
GL Ag vs. HCF Ag	0.442	0.04	0.36-0.52	10.99	< 0.0001
HCF Ag vs. Pscs Ag	0.442	0.04	0.36-0.52	10.99	< 0.0001
Pscs Ag vs. LL Ag	0	0	0	-	> 0.05
LL Ag vs. HCF Ag	0.442	0.04	0.36-0.52	10.99	< 0.0001
GL vs. Pscs Ag	0	0	0	-	> 0.05

SE: standard error; AUROC: Area under receiver operator characteristic curve; CI: confidence interval.

Table 7: Area under the curve, sensitivity, specificity and accuracy for different HC prepared antigens

	LL Ag	GL Ag	HCF Ag	Pscs Ag
Cut-off	> 0.318	> 0.4	> 0.23	> 0.321
AUC (95% C.I)	100	100	0.56	100
Sensitivity(95% C.I)	100 (83.16-100)	100 (83.16-100)	100 (80.49-100)	100 (83.16-100)
Specificity(95% C.I)	98.00(94.27-99.59)	98.00(94.27-99.59)	55.8 (47.31-63.58)	98.00(94.27-99.59)
Accuracy (95% C.I)	98.24 (94.93-99.63)	98.24 (94.93-99.63)	60(52.22-67.42)	98.24 (94.93-99.63)

AUC: Area under receiver curve, CI: confidence interval, GL: Germinal layer, HCF: Hydatid cyst fluid, LL: laminated layer, Pscs: Protoscolices.

Table 8: Intra-class correlation for quantifying the four antigens agreement.

Antigens	LL	GL	HCF	Pscs	Overall agreement among the four antigens
LL	-	0.65(0.52-0.74)***	-0.21(-0.64-0.1) *	0.72 (0.62-0.79)***	0.21 (0.01 – 0.39)*
GL	-	-	-0.98(-1.67- - 0.46) *	0.55 (0.39-0.67)**	
HCF	-	-		-0.995(-1.7- -0.48) *	
Pscs	-	-	-	-	

*: poor agreement degree *** good agreement degree,** moderate agreement degree, GL: Germinal layer, HCF: Hydatid cyst fluid, LL: laminated layer, Pscs: Protoscolices.

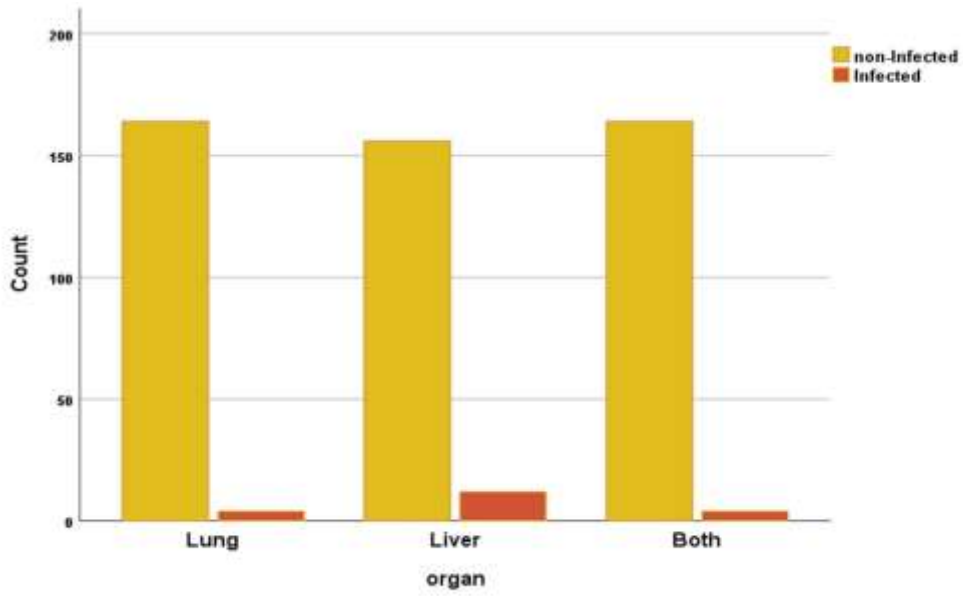

Fig. 1: Prevalence of HC among different breeds and organs of slaughtered cattle

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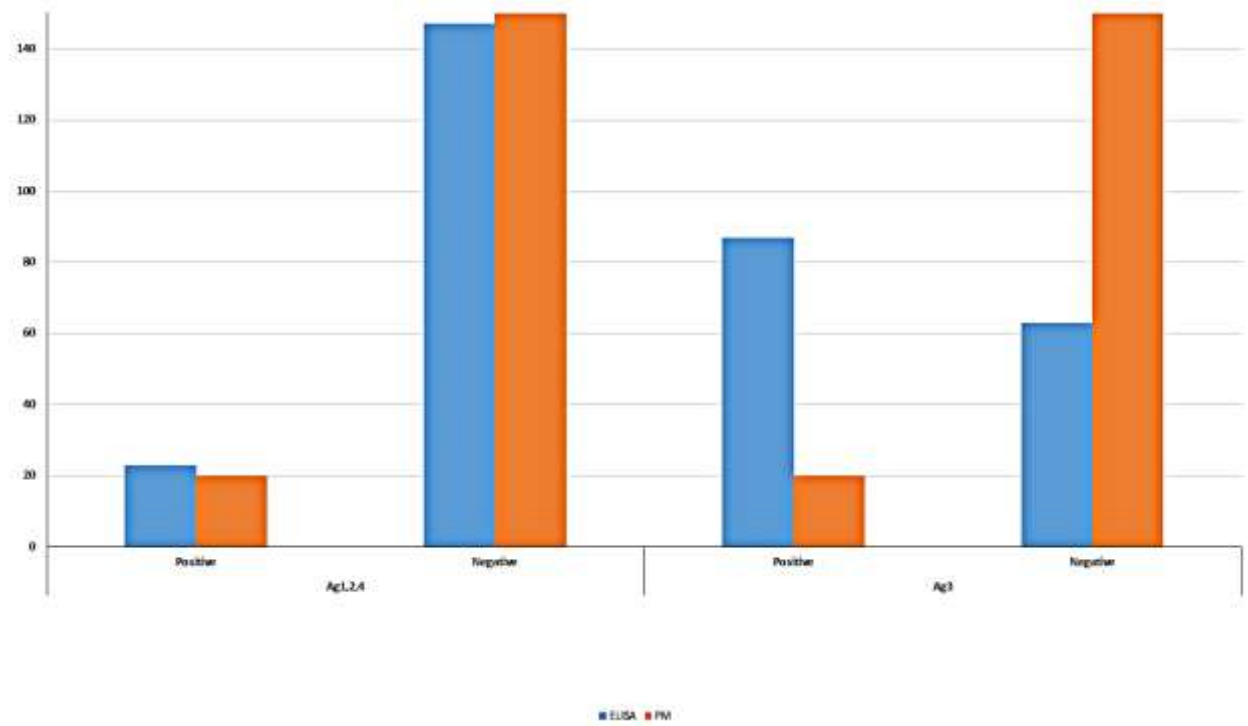

Fig. 2: Comparison of positivity and negativity percentages for different used antigens using ELISA and PM examination methods, Ag1 (LL), 2(GL), 3(HCF), and 4(Pscs).

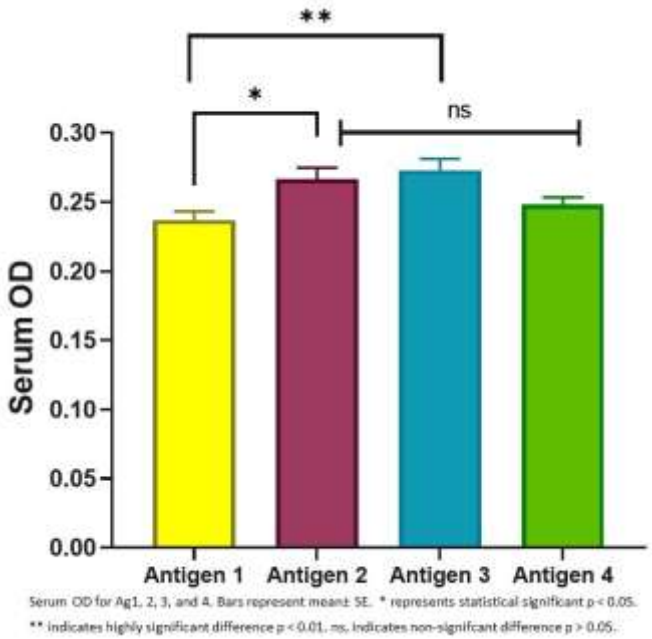

Fig. 3: The results of serum samples analyzed by ELISA with Ag1 (LL), Ag2 (GL), Ag3 (HCF), and Ag4 (Pscs) were represented through the mean and standard deviation (SE).

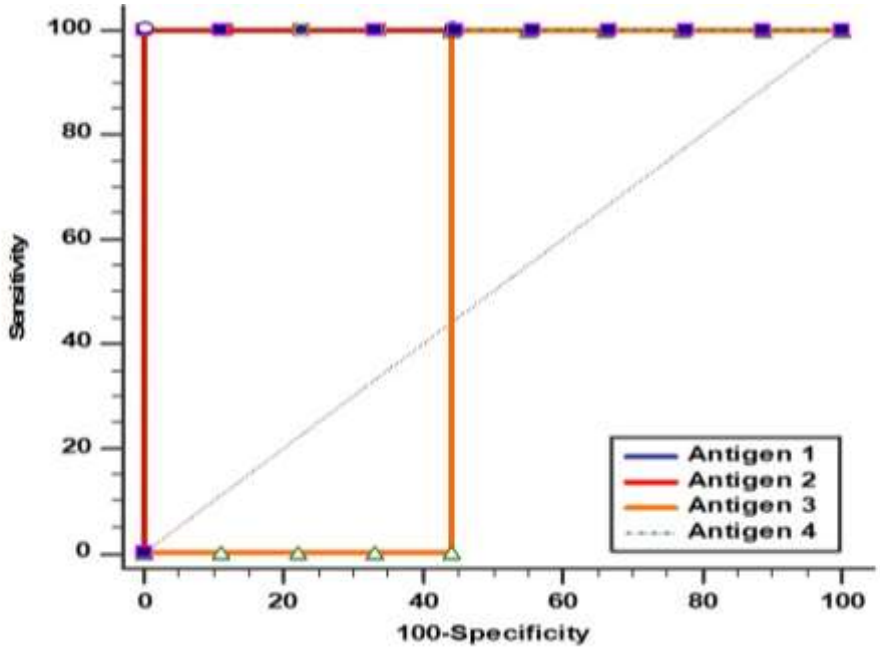

Fig. 4: Area under the curve, sensitivity, and specificity for different prepared Ags compared to the PM examination (Ag1 (LL), Ag2 (GL), Ag3 (HCF), and Ag4 (Pscs))

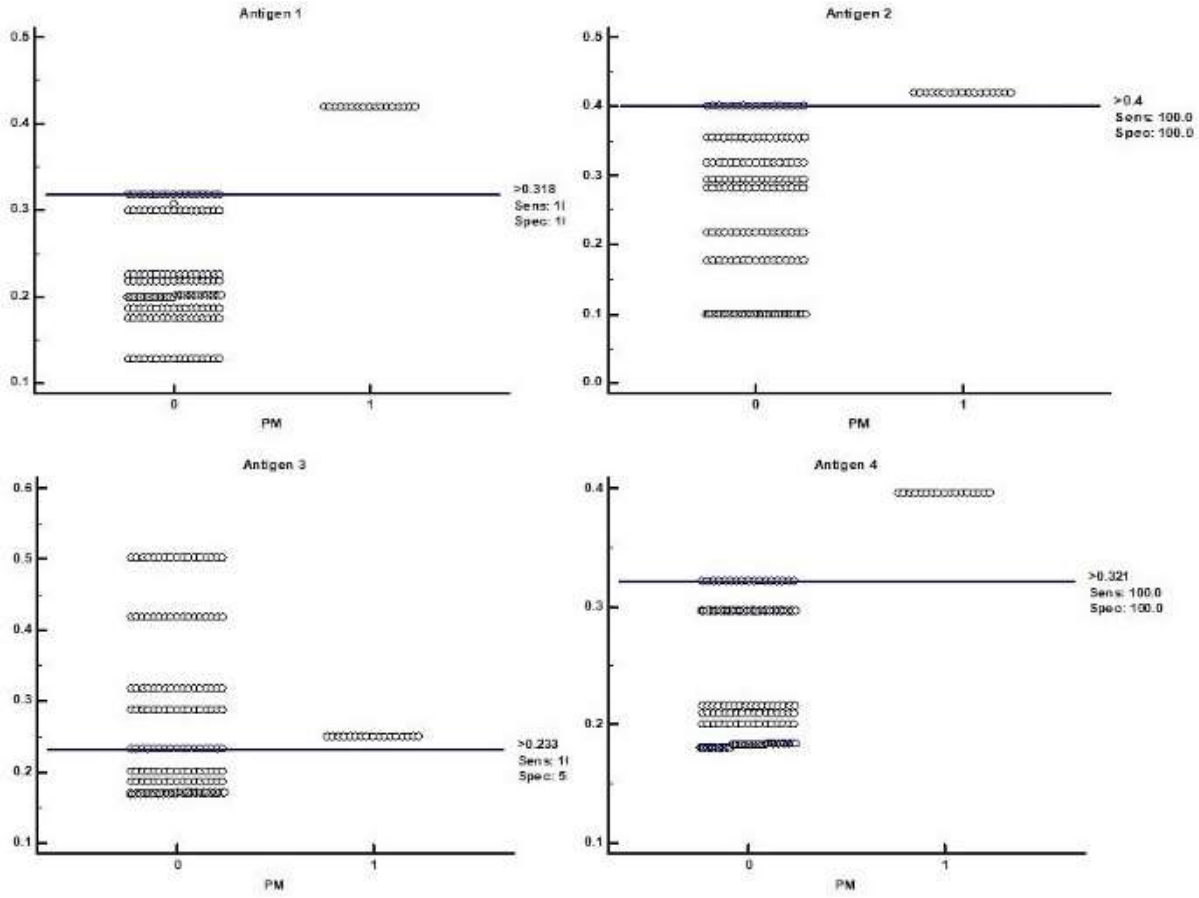

Fig. 5: Classification performance of used Ags in ELISA compared to PM results. 0 denotes negative results, 1 denotes positive results. The horizontal line represents the best cut off (> 0.318) produced by Youden index. Sens. Sensitivity, spec. specificity. .

Discussion

Cystic echinococcosis (CE) is an important neglected disease. CE is not an obvious problem for farmers, owing to its asymptomatic nature in infected hosts. In addition, the infection with adult worms was un- noticeable in dogs as final host infection. Its control depended upon regular dosing of dogs with anthelmintic, reduction of stray dog populations, preventing their access to dogs via consumption of condemned infected organs, hygienic improvement of livestock slaughter practices **(Dar and Alkarmi 1997 and Khan et al. 2019).**

The current study recorded 11.76 % prevalence rate for hydatid cysts in the examined cattle in their liver and lungs. It reached 10.81% and 12.03% for slaughtered native and crossbreed cattle, respectively. The cysts mainly lodged in the examined liver (7.06%), followed by lung and both of them to be 2.35%. This might be due to a great blood capillary suppling liver in comparison with other organs. In addition to, the passively passage of oncospheres to different body portions via portal circulation, which considered the nearest to liver **(Abdel-Baki et al. 2018).**

Nearly Similar rates were reported in China to be 9.62% by **(Yang et al. 2022)** and 12.83% in Pakistan by **(Mahmood et al. 2022)** where the highest rate reached 35.71% in liver followed by 33.33% in lungs and 20% for both of them.

Lower rate was recorded in Iraq to be 1.84% by **(Jawad et al. 2018)** where the examined cattle showed a higher infection rate in lung (1.22%) than liver (0.61%). Also, **(Amer et al. 2018)** reported 2.76% for cysts in cattle in Saudi Arabia. In Romania, **(Dărăbuș et al. 2022)** recorded 2.45% rate mainly lodged in the lungs. This reflected an improvement in the sanitary–veterinary control measures at the farm levels , improvements in canine population management programs and educating owners (via veterinarians) to deworm their dogs.

Higher rates were reported in Pakistan to be 14.4% by **(Khan et al. 2023)** dominated mainly in lungs (14.1%) and then, in liver to be (5.5%), in China reached 17.27% by **(Fan et al. 2022)** mainly located in liver, in Ethiopia reached 17.9% by **(Mathewos et al. 2022)** with rate of 8.3%, 5.4% for lung and liver, respectively, in Kazakhstan by **(Bulashev et al. 2017)** reached 19% where the lungs were mainly affected (52%) followed by the liver, and then, 24% for both of them (24%).

A very higher rate was obtained in Morocco to be 42.9% by **(El Berbri et al. 2015)** where 30.7% of lungs, 30.0% of liver, and 39.3% of liver and lungs were infected. This might be attributed to different geographical and climatic factors where temperature, rainy, low, and high altitude areas were more suitable for the survival of *E. granulosus* eggs **(Fan et al. 2022; Yang et al. 2022)**. In addition, **Dărăbuș et al. (2022)** suggested the higher rates might be caused by more chance for contact with dogs as final hosts, unperfected control of stray dogs, and absence of national control measures against hydatid cyst infection.

Diagnosis of hydatid cyst (HC) in intermediate hosts mainly based upon PM examination, where the infection appeared asymptomatic especially in the early stages, and so it is necessary to use ElISA in the current study as a diagnostic tool for antibody detection in the serum of the examined cattle. Also, the current study used four prepared antigens form HC in lung and liver of slaughtered cattle in abattoirs. Many previous studies used ELISA test for the diagnosis of HC incidence, but very little data is known about using and evaluating different prepared HC Ags in cattle.

The obtained prevalence rate by ELISA reached 13.5% against 11.76% rate obtained by PM examination. This might be due to presence of small sized cyst which neglected during PM examination or early stages of infection with HC.

Nearly similar rates were noticed in Kazakhstan by (Bulashev et al. 2017) to be 9%, 10%, and 14% against s-ELISA/ES-Ag, s-ELISA/S-Ag, and i-ELISA/S-Ag, respectively, in Pakistan by (Khan et al. 2023; Aziz et al. 2020) to be 19.6% and 24.2% against crude BHCF antigen. Higher rates were reported as the following; in Turkey by (Simsek et al. 2005), to be 63.3% against partially purified HCF Ag from sheep, in Moldova by **(Chihai et al. 2012)** to be 70%, and in Ethiopia, **(Golassa et al. 2011)** used HCF Ag and obtained 44.02 % rate. The obtained higher rate might be resulted from more sized population of stray dogs in close contact with human and livestock animals, poor hygienic conditions for disposal of condemned offals contained HC **(Qingling et al. 2014).** Also, **Moje et al. (2014)** referred high rates to the fear of owners from inducing multiple incisions in their slaughtered carcasses during PM examination of HC to avoid decreasing the market value of meat, and unofficial animal slaughtering outside abattoirs without veterinary inspection and proper hygienic facilities. In addition, the presence of different animal breeds as shown in the current study and previous study by **(Simsek et al. 2005)** where a higher rate was recorded in crossbreed cattle.

The obtained results showed high significant differences between the four used Ags by Tukey's test where GL, HCF, and Pscs Ags showed higher values in comparison with LL Ag. The diagnostic accuracy of using ELISA was excellent (100%) for LL, GL, and Pscs Ags while, it was bad (56%) for HCF Ag. Like our study, **(Abo-Aziza et al. 2020)** used different HC antigens but from camel and recommend using HCF Ag for antibodies detection which contradicted our obtained results in cattle.

The difference between the obtained statically analyzed data and previous studies might be due to using different Ags from different breeds of cattle and type of cyst (fertile, calcified or sterile) where, **Bulashev et al. (2017)** refereed low sensitivity to the presence of sterile and calcified cysts, relatively high specificity and high antibody titer were refereed to fertile cysts incidence.

Previous studies reported the efficiency of HC Ags extracted from lung and liver of infected cattle to diagnose human cystic echinococcosis where, **Irabuena et al. (2000)** used BHCF Ag, and **Bulashev et al. (2017)** used the excretory-secretory antigen (ES-Ag) and somatic antigen (S-Ag). **Bulashev et al. (2017)** reported 48%, 52% and 62% sensitivity for s-ELISA/ES-Ag, s-ELISA/S-Ag and i-ELISA/S-Ag, respectively. However, their specificity reached 80%, 73%, 53%, respectively. **Golassa et al. (2011)** used fertile HCF Ag from naturally infected sheep for the serological diagnosis of hydatidosis in cattle by ELISA. The diagnostic accuracy, specificity and sensitivity reached 87.6%, 83.3%, and 96.0%, respectively. **Simsek et al. (2005)** used partially purified HCF Ag from sheep HC to diagnose cattle infection by ELISA and reported a high sensitivity (63.3%) readings against other cross reacting parasitic Ags. **Bulashev et al. (2017)** reported that the sensitivity of s-ELISA/ES-Ag, s-ELISA/S-Ag and i-ELISA/S-Ag reached 48%, 52% and 62%, respectively, in the serum of examined cattle. **Rafiei et al. (2017)** reported HCF and Pscs Ags had better performance than LL Ag. In spite of LL glycan bands constituent which stimulated the body to produce an immune response against HC infection. **Mousa et al. (2015)** demonstrated higher sensitivity of Pscs Ag than HCF Ag, while their specificity and diagnostic efficacy were lower than HCF Ag. This might be due to presence of more specific-proteins in HCF Ag than in Pscs Ag. Also, **Fotoohi et al. (2013)** recorded better performance of HCF in comparison with secretory / excretory and somatic antigens from sheep PScs HC against human cystic echinococcosis. **El-Kattan et al. (2020)** evaluated different HCF Ag in ELISA where the sensitivity for crude HCF Ag was 82.76% while it was 79.31 %

for the partially purified HCF Ag. On the other hand, their specificity reached 62.5 % and 75.0 %, respectively. **Hassanain et al. (2021)** used different antigens (HCF, GL and Pscs) extracted from slaughtered camels against of human and camel cystic echinococcosis. ELISA readings proves that HCF and GL Ags from camels were higher than those extracted from human cysts. They exhibited 100% sensitivity for both camel and human origin Ags and 78.26% and 76.47% specificity for both, respectively. The latter proved the closed binding reactivity between camel and human strains of HC. This made the previous study to recommend usage of animal origin HC Ag as a potent immunodiagnostic Ag for human cystic echinococcosis.

Conclusion

Our current study revealed that antibody detection assay is a sensitive approach for the diagnosis of hydatid cyst in cattle. This study recommended using LL GL, and Pscs Ags as accurate, sensitive, immunodiagnostic Ags for screening HC infection in livestock animals.

From previous explained results, the following recommendations are required to decrease their risk on general public health and economic losses:

Regular deworming of pet dogs and control of stray dogs.

Public awareness creation about transmission, control of HC and its public health hazards.

Proper hygienic disposal of condemned carcass/organs either by burning or burring.

Collaboration between veterinarians and public health workers in prevention and control measures against HC transmission.

Proper food and personal hygiene especially, those in close contact with dogs.

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الملخص العربي

التقييم المصلي لداء أكياس الهيداتيد في الأبقار باستخدام مستضدات مختلفة من السائل الخام داخل الأكياس المائية، والرؤيسات الأولية، والطبقة الجرثومية الداخليه، والطبقة المغلفه الخارجية.

منى محمد إبراهيم عبدالرحمن[1]*-عبدالله فكري عبدالله [2]

-عبدالسلام الديدامونى حافظ[2] -رانيا حلمى محمد شطا[2] - رشا محمد البيومى[2]

قسم الطفيليات-كلية الطب البيطرى- جامعة الزقازيق[1]

قسم صحة وسلامه وتكنولوجيا الغذاء -كلية الطب البيطرى- جامعة الزقازيق[2]

يعد داء الأكياس العدارية (CE) من الأمراض الطفيلية حيوانية المصدر الأكثر انتشارا على مستوي العالم والذى تسببه المرحلة اليرقي *Echinococcus granulosus*.والجدير بالذكران هذا المرض ينتشربشكل كبير في البلدان ذات المناخ المعتدل، مما يؤدي إلى انخفاض وزن الذبائح، وتراجع الإنتاجية، وإتلاف الأعضاء المصابه. قد تبؤ العدوى تحت السريرية دون ظهور أعراض في العوائل النهائية بشكل كبير، بينما تختلف الأعراض في العوائل الوسيطة وفقًا للأعضاء المصابة، وموقع تواجدها، ودرجة العدوى حيث انها قد تسبب قصورًا كبديًا واضطرابات هضمية في حاله أصابه الكبد، بينما قد تؤدي إلى ضيق التنفس عند إصابة الرئتين. يعتبر الفحص ما بعد الذبح في المسالخ الطريقة الرئيسية للكشف عن داء الأكياس العدارية، مما يجعل التشخيص صعبًا ويزيد من خطر انتقال العدوى .لذا فقد هدفت الدراسة الحالية إلى تقييم التشخيص المصلي والأهمية الإحصائية لاستخدام مستضدات مختلفة محضرة من الأكياس العدارية مقارنة بطريقة الفحص ما بعد الذبح حيث تم جمع عينات من مصل الدم والأكياس المائيه من الأبقارالتى تم ذبحها في المسالخ، وتحضيرها للفحص من خلال قراءة قيم الكثافة الضوئية لعينات الامصال باستخدام اختبارالمقايسه الامتصاصية المناعية للانزيم ، ثم تحليل النتائج التي تم الحصول عليها إحصائيًا.وقد وجد أن معدل الانتشار حسب فحص ما بعد الذبح وصل الى ١١,٧٦ %، مقابل ١٣,٥% تم اكتشافها بواسطة اختبار المقايسه الامتصاصية المناعية للانزيم وأيضا كان معدل الإصابة الأعلى في الكبد (٧,٠٦%) مقارنة بالرئتين (٢,٣٥%). وأظهرت النتائج فروقًا معنوية كبيرة بين المستضدات الأربعة المستخدمة، حيث أظهرت مستضدات السائل الخام داخل الأكياس المائية، والرؤيسات الأولية ، والطبقة المغلفه الخارجية قيمًا أعلى مقارنة بمستضد الطبقة الجرثومية الداخليه كما بلغت دقة التشخيص لاختبارالمقايسه الامتصاصية المناعية للانزيم تقييم ممتاز (٩٨,٢٤%) لمستضدات والرؤيسات الأولية ، والطبقة المغلفه الخارجية والطبقة الجرثومية الداخليه بينما كان ضعيف (٦٠%) لمستضد السائل الخام.لذا فقد أوصت الدراسة الحالية باستخدام مستضدات مستضدات السائل الخام داخل الأكياس المائية، والرؤيسات الأولية ، والطبقة المغلفه الخارجية المستخلصه من الأكياس العدارية في الأبقار لما لها من قوة تشخيصية مناعية حساسة لفحص داء الأكياس العدارية.