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Improved Dot ELISA Technique for Diagnosis of Some Tissue Parasites Infecting Donkeys

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Abstract: After fractionation of fertile *Hydatid* cyst fluid antigens (FHCFAg) and *Sarcocystis* sp. bradyzoites antigens (SBAg), The bands at MW of 38 KDa, 36 KDa and 33-29 KDa were reacted specifically versus sera of *Hydatid* cysts (HC) infected donkeys as well as Sera of FHCFAg vaccinated rabbit hyper-immune sera (RHIS) using enzyme linked immuno-transfer blot (EITB) technique. By the same way, the bands at the level of 63 KDa, 49 KDa, 36 KDa and 30 KDa in the fractionated SBAg were proved as specific bands versus sera of donkeys naturally infected with *Sarcocystis* sp. and SB Ag vaccinated RHIS. Testing the value of these fractions in diagnosis of HC infections using dot ELISA revealed that all of the above bands proved absolute sensitivity (100%) versus the dotted infected donkey sera as well as RHIS. The degree of color darkness was high for the fraction of 38 KDa versus all of the tested sera. In the same time all of SBAg-EITB specific fractions proved absolute sensitivity (100%) in capturing of their specific Ab from sera of naturally infected donkeys with *Sarcocystis* sp. as well as in RHIS, by the same level of darkness. None of these fractions reacted versus control non infected donkeys or rabbit sera using dot-ELISA. In FHCF Ag, fraction of 38 KDa appear highly specific (100%) with high validity % versus sera of *Sarcocystis* sp., *Fasciola* and *Rhinoestrus* infected donkeys. By the same way, SB Ag fractions showing specificity 80% versus sera of HC and *Fasciola* infected donkeys. But they showing absolute specificity (100%) versus sera of *Rhinoesterus* infected donkeys with validity % reached to 93.3% using dot-ELISA technique. Same results were obtained in comparing the data of HC infection obtained after P.M inspection with that obtained via detection of the parasite Ab in their sera using this modified Dot-ELISA technique in pre-scarified group of donkeys. Dotting of serum samples on previously identified specific protein fractions introduce easily applicable diagnostic technique take the advantage of EITB technique and ELISA.
Key words: Dot-ELISA, donkeys, EITB, *Hydatid* cyst, *Sarcocystis* sp.

1. Introduction

During the last decade, more progress was achieved in diagnostic techniques aiming to describe an accurate easily applicable method suitable for parasitic infection in human and animals. Several modifications were recorded for serological techniques to increase its accuracy and specificity. Really not all of these techniques considered of special values as field applicable test as it usually unsuitable for large number of samples (PCR) more or less expensive and time consuming (EITB).[1]

ELISA described as an easily applied test suitable for evaluating large number of samples smuiltanuosly[2,] but its accuracy depends mainly on degree of purity and specificity of the used antigen[3].

Dot ELISA used as another modification of ELISA technique it was done using very minute amount of sera and antigen. With the ability of extracting specific protein fraction after EITB, Dot ELISA can used as an accurate test gather the benefits of ELISA and that of EITB[4].

Cystic echinococcosis (CE) or Hydatidosis (HD) is recognized as an important worldwide distributed disease from the clinical, economical and zoonotic point of view. The disease in man and other intermediate hosts caused by ingestion of food or water contaminated with eggs of *Echinococcus granulosus*[5]. There is no accurate test able to diagnose tissue parasites especially in animals[6]. Even with using of high specific test such as EITB, selection of antigens and its degree of purity considered as an important point in obtaining an accurate results exclude cross reaction with other related parasites[3].

Fertile *Hydatid* cyst fluid (FHCFAg) was previously described as the most preferable and valuable antigen extracted from fertile HC for immuno-diagnosis of the disease. This antigen is a complex mixture of several antigens derived from the metabolism of the parasite together with many components from the host[7,8]. Fractionation of this antigen using SDS-PAGE and identification of specific bands using EITB technique, demonstrated the bands fractions at the level of 29 KDa and 34 KDa as high specificity than sensitivity[9]. Moreover, fractions of 65 KDa, 26 KDa, 18KDa, 16 KDa, 12 KDa and 8 KDa appear as more specific for detection of IgG antibodies of echinococcosis in general using ELISA technique[10,3].

Sarcocystosis is wide spread disease infect muscles of food producing animals in Egypt. Its incidence was 10.6% and 69.5% in buffaloes of 2 & over 7 years old (by macro & microscopic techniques), but it reached to 19.33% and 88.5% in the above age groups respectively using dot-ELISA technique[11]. After fractionation of *Sarcocystis cruzi* bradyzoites and testing the diagnostic efficacy of the obtained fractions using EITB technique, seven protein bands at molecular weight of 37, 44, 53, 57, 94, 113 and 215 KDa, proved to be specific in diagnosis of infection in suspected and control serum samples[12,13].

Nowadays, fractionation of proteins using SDS-PAGE and EITB technique are important in detection of specific diagnostic parasitic fraction[14]. With the limitation of EITB technique as field, applicable test, development of new technique based on the valuable product of EITB and able to be applied for large number of samples in the same time, considered as an important direction toward characterization of special easily applicable field test such as Dot-ELISA.

For this reason the present study aiming to develop a modified Dot EISA technique based on dotting of the sera on previously EITB identified specific protein fraction from fertile *Hydatid* cysts and *Sarcocystis* sp. of donkeys. Aiming to estimate the value of these fractions in diagnosis of infection using this modified Dot ELISA technique.

2. Materials and Methods

2.1. Fertile *Hydatid* cysts fluid antigen (FHCFAg) preparation

Hydatid cysts (HC) were collected from freshly slaughtered camel lungs (from Cairo slaughter abattoir). They examined from the aspect of viability as well as sample from their fluid was aspirated and microscopically examined for the presence of protoscolices. The FHCFAg was prepared according to [15]where the fluid was collected, clarified by centrifugation at 5000 rpm for 15 min at 4°C, dialyzed against 5mM Tris-Hcl (pH 7.4) for 48 hr at 4 °C. Its protein content was determined by method of [16] then it was allocated into 1ml vial and stored at –20 °C until use.

2.2. Sarcocystis sp. bradyzoites antigen (SBAg.)

According to[17], bradyzoites of *Sarcocystis* sp. were extracted from macroscopic cysts of natural infected donkeys' esophagus by crushing in 0.01M phosphate buffered saline (PBS) pH 7.4. After washing by centrifugation, the bradyzoites were ruptured in few amount of PBS by repeated freezing and thawing (3 times). The contents were sonicated using "Cole parmer ultrasonic Homogenizer (under 150 watt interrupted pulse output at 50% power cycle in ice bath). The suspension was centrifuged at 10.000 rpm at 4 °C for one hour. The supernatant was collected and dialyzed overnight in refrigerator against PBS, pH 7.2 using a dialysis membrane (6000 to 8000 molecular weight cut off), its protein contents was measured, allocated and store as before.

2.3. Electrophoretic fractionation of antigens

The prepared antigens were resolved using 1.5 mm thickness, Sodium dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS- PAGE) according to[18] in 12 % polyacrylamide gel slabs in Tris-glycine buffer, pH 8.3 under reducing conditions. The stacking gel consisted of 5 % acrylamide prepared in 12.5 mM Tris-HCL buffer (pH 6.7) (Sigma chemical Co.). Pre-stained low molecular weight (MW standard was employed (Sigma SDS-100B). The comb was adjusted as one small well for standard and one large for the sample.

2.4. Transfer of protein fractions onto nitrocellulose (NC) sheet

Electrophoresis transfer of fractionated proteins onto NC was performed according to[19] using transfer buffer (25 mM tris-base, 192 mM glycine, 20% (v/v) methanol at pH 8.3). Transferring was carried out at 10V, 100 mA overnight at 4°C. The sheet was dried and stored in freezing until use.

2.5. Determination of specific protein fractions using EITB

A longitudinal NC strips (15 x 0.5 cm) containing the fractionated antigen were cut out and allowed to react versus known positive and negative control serum samples at 1:100 dilution, 0.5ml of sera/ strip via Western-blot assay (EITB) according to[19]. Horseradish peroxidase conjugated anti-protein A (Sigma Immunochemicals) was used as conjugate at 1:1000 in 3% BSA/PBS. The used substrate is 4-chloro-1-naphthol. Fractions that react versus reference positive sera and at the same time did not react versus negative control one considered as specific protein fractions (plate 1).

These strips of NC were returned back to its original position on NC sheet for determination to the sit of the specific reacted KDa protein fractions on the whole NC sheet.

2.6. Dot-ELISA Technique

The technique described by[20] after modification was adopted. The level of specific protein fractions were determined visually on the whole non treated NC sheet. Using the pencil longitudinal and transverse lines was drawn on the sheet before and after each specific KDa protein fraction (Plate1). Before testing the selected serum samples, the non-specific binding sites were blocked by immersing the whole NC sheet in 3% BSA in PBS-T for 30 min. After 3 time wash in PBS-T, The sheet was left to dry then 2µlof the tested sera was dotted on each determined square on NC and left to dry at 56°C for 10-15 min. then washed as before. Four µl of anti-Protein A peroxidase conjugated (Sigma) diluted at 1:1000, was doted to each well and left to dry at room temperature. After washing, the sheet was immersed in 5 ml of freshly prepared substrate solution (4-chloro-1-naphthol 340 µg/ml substrate buffer with 0.03% hydrogen peroxide solution). Color developed within 15 min., the sheet was washed with water and air-dried. The intensity of the blue-purple color was judged by the naked eye and evaluated in comparison with the reference control and tested sera. Sensitivity was calculated as the number of true positive / (True positive + False negative). While specificity calculated as the number of true negative / (True positive + False negative) [21]. The NC sheet containing the specific fractions can be used directly or after storing at –20 °C to be use when needed.

Validity of the test was estimated according to[22] by calculating the mean of sensitivity and specificity (Validity = mean sensitivity + mean specificity/2).

2.7. Examined serum samples

The following serum samples were selected from large number of samples collected during other relate work. The samples selected after PM inspection as well as from animals of faces free from any other parasitic infection as the following:

1-Ten serum samples from HC natural infected donkeys.

2-Four serum samples from *Sarcocystis* sp. infected donkeys.

3-Five serum samples from *Fasciola* sp. infected donkeys.

4-Five serum samples from *Rhinoesterus* sp. infected donkeys.

5-25 serum samples from donkeys of parasitic free fecal samples have available PM data. (These samples were included in sero-diagnosis for anti-HC antibodies by ELISA techniques and their data was confirmed by PM examination for slaughtered animals or sonographic findings for human to confirm the results.)

6-Ten serum samples from parasitic free donkeys after fecal examination and PM inspection

2.8. Hyper-immune sera

Rabbit hyper-immune sera (RHIS) were raised versus FHCFAg and SB Ag according to[23]. Two (2 months old) white New Zealand rabbits were bled for negative control sera then injected with 1.2 mg protein for each antigen, mixed in an equal volume of mineral oil subcutaneously. After 3 weeks, 3 consecutives injections of 0.4 mg protein antigen in equal volume of oil were given intramuscularly at biweekly intervals. Rabbits were bled from the ear vein for serum collection 10-14 days after the last injection. The collected sera were stored at –20°C until used.

3. Results

The data in table (1) and Plate (1) demonstrate different protein fractions diagnosed after treatment of fractionated FHCFAg and SBAg on NC strips versus positive and negative donkeys and rabbit sera using EITB technique. The data demonstrate that treatment of NC strips carrying FHCFAg versus severely HC natural infected donkey sera (proved after PM inspection) revealed 6 reacted fractions corresponding to MW standard at the level of 135 KDa, 68 KDa, 38 KDa, 36 KDa, 18 KDa and 12 KDa, were diagnosed after treatment of corresponding strips versus negative non-infected healthy donkeys. By the same way, treatment of NC strips carrying the fractionated SBAg versus *Sarcocystis* sp. natural infected donkey sera, revealed 10 bands corresponding to the MW standard at the level of 135 KDa, 100 KDa, 76 KDa, 63 KDa, 49 KDa, 36KDa, 30 KDa, 23 KDa, 18 KDa and > 11 KDa. Most of these bands except that at MW level of 63 KDa, 49 KDa 36 KDa and 30 KDa were recorded at treatment of corresponding NC strips versus control healthy non infected donkeys (table & plate 1).

Collectively, treatment of fractionated FHCFAg and SBAg on NC strips revealed that the fractions at the level of 38 KDa, 36 KDa and 33-29 KDa in FHCFAg and that at the level of 63 KDa, 49 KDa, 36KDa and 30 KDa in SBAg considered the main specific parasitic fractions in both antigens.

Testing the value of these fractions in diagnosis of infections by both parasites using dot ELISA revealed that the 3 specific fractions diagnosed via EITB for the FHCFAg at the level of 38 KDa, 36 KDa and 33-29 KDa proved absolute (100%) sensitivity versus the dotted infected donkey sera as well as versus RHIS till 1:300 dilution (table 2). The degree of colour darkness was high for the fraction at 38 KDa versus all of the tested sera, flowed by the fraction of 36 KDa as it weakly reacted versus high diluted RHIS, (plate 1).

Concerning the sensitivity of the tested EITB specific SBAg (at the level of 63 KDa, 49 KDa, 36 KDa and 30 KDa), All of these fractions proved absolute sensitivity (100%) in capturing of their specific Ab from sera of *Sarcocystis* sp. natural infected donkeys as well as in RHIS at 1:100 dilution, by the same level of darkness. None of these fractions reacted versus 1:300 RHIS or versus control non infected donkey or rabbit sera using dot-ELISA (Plate 1 & table 3).

Concerning specificity of these fractions using dot-ELISA, the fraction corresponding to 38 KDa of FHCFAg appear highly specific as it did not react by any degree when treated versus sera of *Sarcocystis* sp., *Fasciola* sp. and *Rhinoestrus* sp. infected donkeys. While the other two fractions (36 KDa & 33-29 KDa) showing 86.66% and 78.33% specificity (table 3). This affected on the degree of validity % which decreased from 100% to 93.3% and 89.15% for the above 3 fractions respectively.

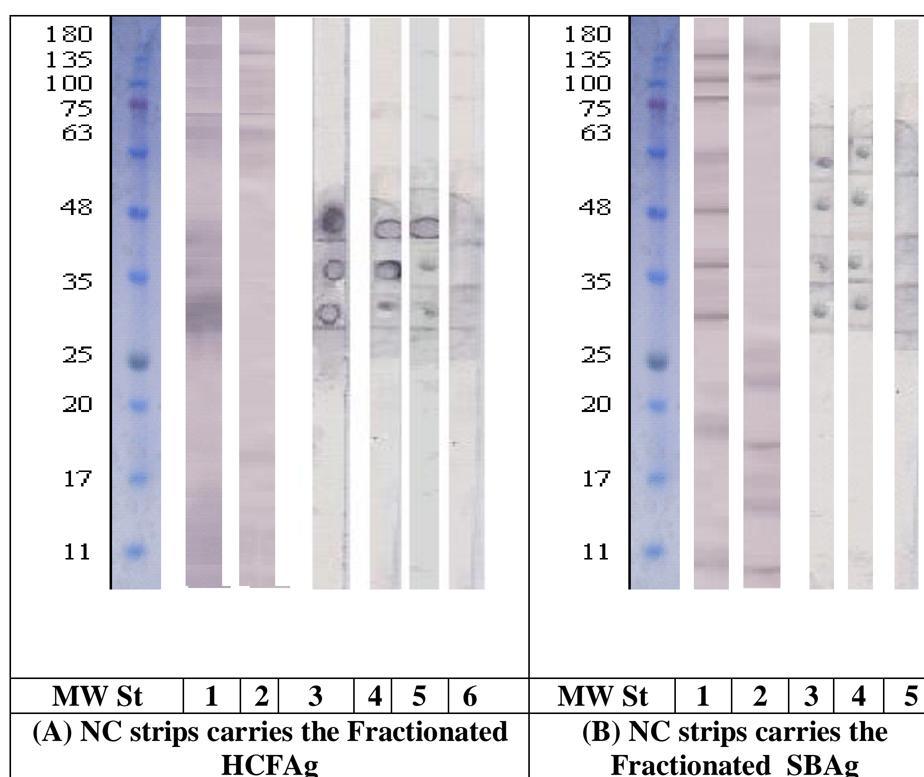
In the same time, all of SB Ag fractions showing the same degree of specificity (80%) versus sera of HC and *Fasciola* sp. infected donkeys. In the same time they showing absolute (100%) specificity versus *Rhinoesterus* sp. infected sera (table3). This reducing their mean specificity into 86.6% for all of the tested fraction. In the same time this decreasing the validity % of the test to 93.3% with similarity for all of the selected fractions. (Table, 3).

The diagnostic efficacy of the improved dot-ELISA technique was evaluated via comparing the data obtained after P.M inspection with that obtained via detection of the parasite Ab in their sera. The test was done on 25 pre-slaughtered over 7 years old donkeys in Giza zoo, as described in table (4). (Applicability of the test). The date proved high sensitivity in diagnosis of HC infection as the test results was closely related to that obtained after PM inspection (44%), 11 animals. While low number 3 (12%) could be infected by *Sarcocystis*

sp. in muscle by naked eye in comparing with 8 (32%) appear positive after testing of their sera using the modified Dot-ELISA technique (table 4).

Table 1: Specific and non -specific protein fractions of FHCFAg and SBAg using Western blot versus reference positive and negative sera

Band No	KDa bands reacted in On NC strips contained			
	Fractionated FHCFAg treated with sera of		**Fractionated SBAg treated with sera of**	
	Infected donkey	**Non-infected donkey**	**Infected donkey**	**Non-infected donkey**
1	135 KDa	135 KDa	135 KDa	135 KDa
2	68 KDa	68 KDa	100 KDa	100 KDa
3	►38 KDa	18 KDa	76 KDa	76 KDa
4	► 36 KDa	12 KDa	► 63 KDa	25 KDa
5	►33-29 KDa		► 49 KDa	23 KDa
6	12 KDa		► 36 KDa	18 KDa
7			► 30 KDa	17 KDa
8			23 KDa	13 KDa
9			18 KDa	> 11 KDa
10			> 11 KDa	



(A) NC strips carries the Fractionated HCFAg

(B) NC strips carries the Fractionated SBAg

Fig. 1: Recognition of fractionated FHCFAg (A) & SBAg (B) on NC strips using EITB technique and reaction of its specific bands using modified Dot ELISA

Lane 1 (A&B) = reacted versus donkey sera infected by each parasite.
Lane 2 (A&B) = NC strip treated by non-infected donkey sera.
Lane 3 (A&B) = Dotted rabbit HI sera versus specific band. (1:100)
Lane 4 (A& B) = Dotted infected donkey sera versus specific band.
Lane 5 (A) = Dotted rabbit HI sera versus specific band. (1:300)
Lane 6 A & 5B = Dotted negative donkey sera versus specific band.

* MW St = Low Molecular weight protein slandered (Sigma)

Table 2: Sensitivity and specificity of EITB Specific FHCFAg protein bands in diagnosis of infection using dot-ELISA technique

History of tested serum samples		No. of tested samples	FHCFAg tested fractions					
			38 KDa		36 KDa		33-29 KDa	
			No. +ve	%	No. +ve	%	No. +ve	%
Sensitivity	RHIS 1: 100	2	2	100	2	100	2	100
	Natural infected Donkeys	10	10	100 %	10	100%	10	100 %
	RHIS. 1: 300	2	2	100	2	100	2	100
	Mean			**100**		**100**		**100**
Specificity	*Sarcocystis* sp. infected donkey	4	0.0	100	0.0	100	1	75
	Fasciola infected donkeys	5	0.0	100	1	80%	1	80%
	Rhinoestrus infected donkeys	5	0.0	100	1	80%	1	80%
	Mean			**100**		**86.66**		**78.33**
Validity (%)			**100 %**		**93.3 %**		**89.15 %**	

* No reaction could be recorded with sera of negative rabbit, healthy donkeys of parasite free faeces.

Table 3: Sensitivity and specificity of EITB Specific SBAg protein bands in diagnosis of infection using dot-ELISA technique

Tested serum samples		No. of tested samples	SBAg tested fractions							
			63 KDa		49 KDa		36 KDa		30 KDa	
			No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%
Sensitivity	Natural infected Donkey	4	4	100	4	100	4	100	4	100
	RHIS. 1: 100	2	2	100	2	100	2	100	2	100
	RHIS. 1: 300	2	0	0.0	0	0.0	0	0.0	0	0.0
	Mean (1:100)			**100**		**100**		**100**		**100**
Specificity	HC infected donkey	10	2	80	2	80	2	80	2	80
	Fasciola infected donkey	5	1	80	1	80	1	80	1	80
	Rhinoestrus infected donkey	5	0.0	100	0.0	100	0.0	100	0.0	100
	Mean			**86.66**		**86.66**		**86.66**		**86.66**
Validity (%)			**93.3 %**		**93.3 %**		**93.3 %**		**93.3 %**	

* No reaction could be recorded with sera of negative rabbit, healthy donkeys of parasite free faeces

Table 4: Diagnostic efficacy of the technique in selected group of Donkeys before and after slaughtering in Giza Zoo. (Applicability of the test):

	Diagnosis by dot ELISA for		PM data, infection by	
	HC infection	***Sarcocystis* sp. infection**	**HC infection**	***Sarcocystis* sp. infection**
No. Examined	25	25	25	25
No. infected	11	8	11	3
% of infection	44 %	32 %	44%	12 %

4. Discussion:

Hydatidosis and Sarcocystosis infections recorded as asymptomatic slowly developed disease there diagnosis in animals is almost made during postmortem inspection. In the same time both parasites having sever adverse effect on the economic values of living animals especially at high level of infection. Moreover, donkeys considered to be one of the neglected reservoirs of both diseases, while carcasses of this animal species are usually exposed to predation by stray dogs, this increasing their role as a source for spreading of these types of parasites[24].

Availability of specific and sensitive diagnostic technique able to diagnose infection in living animal facilitate pre-slaughtering identification of infected animals by the way leads to slaughtering them under special restricted control measures, This approach facilitate control of these tissue parasites and prevent their spreads as they have some zoonotic important[13].

Nowadays, the insensitive and non-specific tests for diagnosis of hydatidosis such as casoni intradermal test, the indirect haemagglutination test and the latex agglutination test have been replaced by the enzyme linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody test and immunoblotting (IB) in routine laboratory application aiming to improve the accuracy of the results and minimize the level of cross reaction with other parasites[6]. Moreover, serological diagnosis of hydatidosis, considered as beneficial tool for diagnosis of infection, but cross-reaction between different parasites still as a questionable point making some difficulties in the accuracy of the results, as their accuracy was affected by degree of purity and specificity of the used antigens. For this reason identification of special antigenic fraction could be improve the accuracy of the used diagnostic technique[7].

EITB is one of the most specific sero-diagnostic techniques but it considered non-practical for current field application in comparison with ELISA technique[25]. While, ELISA is one of the best sensitive sero-diagnostic technique, able to analyze many samples simultaneously[2].

The present study aimed to use the benefits of EITB technique in determination of the most specific and sensitive protein fractions in FHCFAg and SB Ag Then testing the diagnostic efficacy of these fractions on NC for current diagnosis of infection by dotting minute amount of tested sera on the previously determined specific protein fractions.

Treatment of NC strips containing the fractionated FHCFAg and SB Ag on NC strips using EITB technique revealed that the fractions at the level of 38 KDa, 36 KDa and 33-29 KDa in FHCF Ag and that at the level of 63 KDa, 49 KDa, 36 KDa and 30 KDa in SB Ag react specifically versus sera of infected animals RHIS and in the same time did not cross reacted with any of the other tested serum samples, these bands considered to be the main specific and sensitive fractions in both parasites. These results was in agreement with that previously mentioned by[13] and[3].

By dotting the tested and control sera on these fraction after their determination on the crude NC sheet, the obtained data revealed that the 3 specific fractions diagnosed via EITB for the FHCF Ag (38 KDa, 36 KDa & 33-29 KDa) proved absolute (100%) sensitivity and specificity versus the dotted infected and control sera with more color darkness for the at 38 KDa As this fractions were tested for the first time using this modified Dot-ELISA technique. The obtained data was discussed upon the previous published EITB data of the same fractions. From this view the obtained results was agreed with[26], as well as[27], this in the contrary with[28] who mentioned that the fraction of 81, 64 and 44 KDa were immunologically considered the most sensitive fraction for HC of dogs. This difference may be related to the difference in the host sera evaluated.

Concerning the applicability % of these fractions (Validity %) and according to[22], it was reached to 100%, for the first fraction while it was decreased to 93.3% and 89.15% for the other 2 fractions respectively. This was supporting using the fraction of 38 KDa as promising fraction for field use among the suggested improved Dot ELISA technique.

In the same time all of the tested SB Ag fractions react by the same level proved absolute (100%) sensitivity using Dot-ELISA as that previously described using EITB technique. This results came in agreement with[13] and in the contrary with[12]. For testing the specificity of these fractions, sera of donkeys infected by *Fasciola* sp. and *Rhinoestrus* sp. available in the laboratory from other study as well as that of HC and

sarcocystis infected donkeys were used. All tested SB Ag fractions showing 80% specificity versus *Fasciola* sp. and HC infected donkey sera. In the authors opinion and as a results of high incidence of infection by HC and *Fasciola* sp. and in relation to the age of these donkeys, these 2 animals cross reacted with SB Ag selected fractions may be reflect a cases of true unapparent infection by theses parasites. The conditions which did not recorded versus *Rhinoestrus* sp. infected donkeys. This explanation appear close up to the real conditions of the examined animals as evaluating the infection in the selected 25 donkeys and confirming the data with its PM results revealed close relation between presence of HC in PM and presence of their Ab in sera of the examined animals (44%). In following the same condition for *Sarcocystis* sp. infection and as a result of the nature of inspection and the size of muscle cyst of *Sarcocystis* sp., the serological data appear higher (32%) than that obtained after PM inspection (12%) of the sacrified donkeys.

Concerning the demonstrated improvement in Dot-ELISA described in the present work, the study overcome the defect of EITB as it unsuitable for testing large number of samples simultaneously and in the same time take this benefit from ELISA via its suitability for testing large number of samples, Moreover using of minute amount of reagents, sera and saving time known for Dot-ELISA could be considered as an additional benefits..

The essential modification in the new descried technique is using minute amount of tested and control sera, ability of NC sheet containing the purified antigen to store in ready form in freezing. The main modification in this new technique is using 4 chloro-1-naphthol as substrate as in EITB in spite of Ortho-phenyl diamine (OPD) substrate in traditional EISA procedures. The test facilitates examination of large number of samples by the same accuracy of EITB technique and in the same time it can be evaluated by naked eye without the need of special ELISA reader.

Develop of modified Dot EISA method based on dotting of the sera on specific and sensitive protein fraction from fertile *Hydatid* cysts and *Sarcocystis* sp. of donkeys after accurate determination of these fractions using SDS-PAGE & EITB technique, and estimate the value of these fractions in diagnosis of infection using this modified Dot ELISA technique in the form described in the present study appear more practical , fast and suitable for large number of samples in comparison with time consuming EITB whish induce diagnoses of single sample per experiment on long NC strip as sample by sample.

5. Conclusion

For conclusion, Dotting of serum samples on previously identified specific protein fraction introduce easily applicable diagnostic technique tack the advantage of specificity from EITB technique and the benefits of ELISA as screening of large number of samples simultaneously. This Modified Dot ELISA technique open a new field for diagnosis of parasites in pre-slaughtering animal the way that facilitate application of strict control measures at slaughtering of these animals and prevent arrival of their offales to stray dogs, the matter which minimize spread of these diseases around the contact human.

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References

1. Silvana Carnevale, Móonica I. Rodríguez, Eduardo A. Guarnera, Carlos Carmona, Tamara Tanos, Sergio O. Angel. Immunodiagnosis of fasciolosis using recombinant procathepsin L cysteine proteinase. Diagnostic Microbiology and Infectious Disease 2001; 41 (1-2): 43–49.
2. Babba H, Messed A, Masmoudi S, Zribi M, Grillot R, Ambriose-Thomas P, Beyrouti I, Sahnoun Y. Diagnosis of human hydatidosis: comparison between imagery and 6 serologic techniques. Am J Trop Med Hyg 1994; 50 (1):64-68.

3. Taher ES. Does the Origin of *Hydatid* Cyst Antigen Affect Diagnosis of Human Hydatidosis? J Applied Sci Res 2012; 8 (4): 1952-1958.
4. El-Bahy NM. Diagnosis of *Fasciola* infection by Dot ELISA technique using SDS-PAGE eluted excretory secretary (ES) protein fractions. 2nd Veterinary Cong. Fac. Vet. Med., Sadat city, Minufiya, Egypt. 2002; pp. 43- 57.
5. Devi C, Parija SC. Latex agglutination test (LAT) for antigen detection in the cystic fluid for the diagnosis of cystic echinococcosis. Diag Microbiol Infect Dis 2003; 45 (2):123-6.
6. Lightowlers M, Gottstein B. Immunodiagnosis of echinococcosis. Thompson R C A, Lymbery A J. Eds. *Echinococcus* and *Hydatid* Disease. Wallingford, United Kingdom: CAB International 1995; 355-410.
7. Irabuena O, Nieto A, Ferreira AM, Battistoni J, Ferragut G. Characterization and optimization of bovine *Echinococcus granulosus* cyst fluid to be used in immunodiagnosis of hydatid disease by ELISA. Rev Inst Med Trop Sao Paulo 2000; Sep-Oct; 42(5):255-62.
8. Osman M Z. Assessment of role of different hydatid vaccines in protection against animal hydatidosis. PhD Thesis, Dept. of Parasitol. Fac. of Med. Cairo Univ. 2006.
9. Poretti D, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am J Trop Med Hyg 1999; 60 (2):193-8.
10. Dreweck CM, Luder CG, Soboslay PT, Kern P. Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. Trop Med Int Health 1997; 2 (8):779-87.
11. Sabry MA, Shalaby H A. Sarcosporidiosis as an emerging zoonotic disease. J Vet Med Mounifya Univ 2004; 1: 112-119.
12. Mamatha GS, D'Souza Placid E, Suryaarayana VVS. Serodiagnosis of bovine sarcocystosis by enzyme immuno transfer blot (EITB) in naturally infected cattle. J Vet Parasitol 2008; 22: 269-72.
13. Sabry MA, Reda WW. Infection by cyst-producing protozoa among human and food producing animals in Egypt. J Biol Sci 2008; 8 (1):1-7.
14. Rokni MB, Aminian B. Evaluation of the Enzyme-linked Immuno-electro Transfer Blot (EITB) technique using *Hydatid* cyst antigens B/5 and total IgG antibodies in laboratory diagnosis of human hydatidosis. Pak J Med Sci 2006; 22 (2): 127 – 131.
15. Ito A, Ma L, Schantz PM, Gottstein B, Liu YH, Chai JJ, Abdel-Hafez SK, Altintas N, Joshi DD, Lightowlers MW, Pawlowski ZS. Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and *E. multilocularis* protoscolex (EM18). Am J Trop Med Hyg 1999; 60 (2):188-92.
16. Lowery OH, Rosenbrough NA, Randall RG. Protein measurement with folin-phenol reagents. J Biol Chem 1951; 193:265-275.
17. Hong-Moon MH. Serological cross-reaction between *Sarcocystis* and *Toxoplasma* in pigs. The Korean J Parasitol 1987; 25: 188-194.
18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of Bacteriophage T 4. Nature 1970; 227:680-685.
19. Towbin H, Stachelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedures and some applications. Proc Nat Acad Sci USA 1979; 76: 4350-4354.
20. Shaheen HI, Kamal KA, Farid Z, Mansour N, Boctor FN, Woody JN. Dot-ELISA for the rapid diagnosis of human fascioliasis. J Parasitol 1989; 75: 549-552.
21. Attallah AM, el-Masry SA, Rizk H, Ismail H, el-Bendary M, Handoussa AE, el Shazly AM, Arafa MA. Fast-Dot ELISA using urine, a rapid and dependable field assay for diagnosis of schistosomiasis. J Egypt Soc Parasitol 1997; 27 (1):279–89.
22. Sadjjadi SM, Abid H, Sarkani B, Izadpanah A, Kazemina S. Evaluation of ELISA, utilizing native antigen B for Serodiagnosis of human hydatidosis. Iran J Immunol 2007; 4 (3): 167-169.
23. Langley RJ, Hillyer GV. Detection of circulating parasite antigen in murine fascioliasis by two-site enzyme-linked immunosorbent assays. Am J Trop Med Hyg 1989; 41: 472-478.
24. Ahmadi NA. Using morpheme try of the larval rostellar hooks to distinguish Iranian strains of *E. granulosus*. Ann Trop Med & Parasitol 2004; 98 (3): 211-220.
25. Ibarra F, Montenegro N, Vera Y, Boulard C, Flores J, Ochoa P. Comparison of three ELISA tests for sero-epidemiology of bovine fascioliasis. Vet Parasitol 1998; 77: 229-236.
26. Ebtesam MA, Hanan H. Diagnostic value of different antigenic fractions of *Hydatid* cyst fluid from camel and sheep in Kingdom of Saudi Arabia. Acta Trop 2012; 92 (1):17-24.

27. Gadea I, Ayala G, Diego MT, Coat A, Garcia de Lomas J. Immunological Diagnosis of Human *Hydatid* Cyst Relapse: Utility of the Enzyme-Linked Immunoelectrotransfer Blot and Discriminant Analysis. Clin Diagn Lab Immunol 2000 7(4): 549–552.
28. Nagwa I Toaleb, A A Derbala, Eman H Abdel-Rahman. Comparative Diagnostic Evaluation of Crude and Isolated Fractions of *Echinococcus granulosus* in Dogs. Global Veterinaria 2011; 7 (6): 587-592.

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