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MODIFIED DOT ELISA FOR DIAGNOSIS OF ZOONOTIC TOXOPLASMOSIS IN MAN AND SHEEP WITH CLINICAL SUGGESTIVE MANIFESTATIONS

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Abstract

Fractionation of *Toxoplasma gondii* RH strain tachyzoites (TTAg) using SDS-PAGE and identification of its specific bands versus control sera using EITB revealed five group of specific fractions corresponding to molecular weight (MW) level of 32-34 kda, 46kda, 53 kda, 78 kda and 100 kda. Fractions of 32-34 kda were the most sensitive and specific one able to capturing very low level of anti-*Toxoplasma* Ab in the sera and did not cross react with anti-parasites antibodies (Ab) as hydatedosis, *S. mansoni*, HCV, *F. gigantica* and *Cysticercus ovies* as well as control non-infected sera. Thick transverse gel strip containing the fraction of 32-34kda were cut out, its contents were eluted and concentrated, Dotting of 2µl from this fraction on 4mm diameter nitrocellulose (NC) discs for development a modified dot-ELISA technique were evaluated in diagnosis of toxoplasmosis in group of suspected women and sheep. This technique succeeded in diagnosis of the specific anti-toxoplasma Ab in sera of seven suspected sheep, all of them aborted after this. Investigation of some haematological and serum chemical parameters demonstrated significant ($p \leq 0.05$) deviations in the values of WBCs, AST, Urea and Cholesterol, which considered to be important in supporting early diagnoses of infection in suspected cases.

Adaptation of modified Dot-ELISA technique described in this work perform an easily field applied mass screening technique depends on using a minute amount of serum without special equipment. Dot-ELISA by this way gathering the benefits of accuracy of EITB and overcome the cross reaction phenomenon characteristic to ELISA. This improved capability of early diagnosis and control strategy of toxoplasmosis in man and animals.

Key words: Egypt, *Toxoplasma*, Specific fractions, dot- ELISA,Man, Sheep. Clinical parameters

Introduction

Toxoplasmosis is a common zoonotic disease caused by coccidian parasite (*T. gondii)*, which is an obligate intracellular protozoan parasite originate from cats (final host) and infects humans, birds and mammals (Kato *et al*, 2012). Infection in human considered to be oligosymptomatic. Juránková *et al*. (2015) estimated *T. gondii* burdens in sheep tissues, they found that brain was the mostly affected organ with highest parasite loads, others organs were lung, heart kidney and few skeletal muscle, but liver and spleen were negative. Toxoplasmosis causes severe economic losses to sheep industry causing abortions, stillbirth, early embryonic death, fetal resorption or birth of weak kids or lambs as well as reducing fertility (Edward and Dubey, 2013). In humans Toxoplasmosis may cause a febrile illness associated with swollen lymph nodes. In pregnant woman it may cause fetal death, abortions or fetal abnormalities, Also, tachyzoites or tissue cysts may be transmitted to fetus causing blindness (Jones *et al*, 2006) and mental abnormalities in congenitally infected fetus (Wishahy *et al*, 1972).

The clinical manifestations of toxoplasmosis could be confused with many protozoan liver parasites (Cocuz, 2014), as malignant malaria (El-Bahnasawy *et al*, 2010), infantile visceral leishmaniasis (El-Bahnasawy *et al*, 2013) and other infectious diseases.

Specific parasite protein fraction was obtained using a sodium dodecyl sulfate - Polyacrylamide gel electrophoresis SDS-PAGE and Immunotrotransfer Blot (EITB) (Rokni and Aminian, 2006). ELISA is an applicable

test suitable for inspection of large number of samples in the same time, and accuracy depended on purity degree and antigen specificity used (Sun *et al*, 2015). Also, the Dot-ELISA proved to be more accuracy, simple, economic, direct visually read and more rapid microassay which need minute volume of reagents to perform (Swarna and Parija, 2012).

This study aimed to develop a new modified Dot-ELISA technique using accurately identified specific protein fractions of TTAg to diagnose *Toxoplasma gondii* infection in suspected women and sheep. Also, the effect of *T. gondii* on some hematological parameters, liver and kidney functions were evaluated to identify the most characteristic alterations associated with toxoplasmosis.

Materials and Methods

This study was designed and approved by Faculty of Veterinary Medicine, Cairo University's Ethics Committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, as well as that of Faculty of Medicine, Al-Azhar University. This study was conducted from March to September 2017.

T. gondii tachyzoites antigen (TTAg) preparation (Gasbarre *et al*, 1984): *Toxoplasma* (RH) strains tachyzoites were collected from the peritoneal fluid of experimentally infected mice. After washing by centrifugation, tachyzoites were ruptured in few amount of PBS by 3 times freezing and thawing. 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 10.000rpm at 4^{o}C for an hour. The supernatant was collected and dialyzed using dialysis membrane (6000 to 8000 molecular weight cut off) overnight in refrigerator against PBS, pH 7.2. Protein contents was measured after Lowry *et al.* (1951) and stored at -70^{o}C until needed. Fractionation of antigens and transferring of protein: SDS-PAGE analysis of TTAg was carried out under reduced conditions using 12% slab gel accompanied with 5% stacking gel (Laemmli *et al*, 1970). Samples were electrophoresed at a current of 20 mA. The run was calibrated using pertained low molecular weight markers (MW) (Sigma SDS-100B). The comb was adjusted as one small well for standard and one large for the sample. The fractionated proteins in the gel were transferred onto nitrocellulose membrane (NC) at 10V, 100 mA overnight at 4°C (Towbin *et al*, 1979). The sheet was dried and stored in freezing until use.

Identification of specific protein fractions using EITB: A longitudinal NC strips (12x 0.4cm) containing the fractionated TTAg were cut out. Incubate with 3% BSA TNT at 37°C for an hr. to block out the nonspecific binding sites of antibodies. Then, strips were incubated versus known positive and negative control sera at 1:100 dilutions, 0.5ml of sera/ strip for 2 hrs (Towbin *et al*, 1979). After washing, the strips were exposed to 1:1.000 HRP-conjugated antihuman or anti-sheep IgG (Sigma, USA), diluted in blocking buffer, at 37°C for an hr. Strips were transferred to peroxidase substrate (4-chloro-1-naphthol, Sigma). Five positive and 5 negative control sheep' sera were included in each working process. Protein fractions that reacted positively with the positive reference serum, and negatively with the known negative control one were considered as specific fractions (Fig. 1). Molecular weights of specific fractions were identified by the MW standard.

Hyper-immune sera: Rabbit hyper-immune sera (RHIS) were raised against TTAg (Langley and Hillyer, 1989) via initial subcutaneously injection in an equal volume of the mineral oil followed by three consecutive injections and used as known reference sera.

Inspected human and sheep samples: Identified coagulated and non-coagulated blood as well as fecal samples was collected from the following cases:

Human samples: A total of 75 human sera were obtained from El-Hussein and Sayed Galal University Hospitals. These were G1:

Five sera from *Toxoplasma* aborted women. G2: Thirty sera from suspected *Toxoplasma* women collected from adult females in the same families of G1. G3: Sera from five patients infected with surgically proved hydatidosis *granulosus*, ten *Schistosoma mansoni* infected patients (more than 5 EPG), and ten sera from hepatitis C patients. G4: Twenty healthy cross matched control women.

Sheep sera: G5: Twenty sera from *Toxoplasma gondii* aborted sheep.G6: Thirty sera from suspected sheep among the same sheep pen.G7: Five sera from hydatidosis *granulosus* infected sheep, ten from *Fasciola gigantica* infected sheep and five from *Cysticercus ovies* infected sheep.G8: Ten sera from healthy control sheep. Both human and sheep were free from other parasites.

Elution and concentration to gel specific protein fractions: SDS-PAGE analysis of TTAg was carried out as before using thick spacer (2mm). Once gel ran its full length, a peripheral longitudinal strips containing the MW standards and beginning of the fractionated Ag, were cut out, strip was stained by using Coomassie blue (Tsai and Frasch, 1982).

Transverse gel strip containing the fractionated proteins corresponding to MW of 32-34 kda was determined then cut out horizontally across the whole gel. This gel strip was transferred to elution tube membrane 6-8 MW cut off (Spectrum Medical Inc., Los Angeles, CA 900060). Tube was filled with PBS (pH 7.4) and kept in Bio-Rad elution unit at 10V, 100mA overnight at 4^{o}C. Gel material was removed and product was concentrated by using poly-ethylene-glycol versus molecular porous membrane tubing 6-8 MW cut off after Bien *et al.* (2013). The protein contents of theeluted concentrated materials was determined and kept in 1 ml vial at -70°C till needed.

Dot-ELISA Technique: After Shaheen *et al*. (1989) with little modifications, 2µl of the eluted concentrated TTAg protein fractions of 32-34 kda were dotted on circular NC disc. Discs were laid on the bottom of micro-ELISA plate and left to dry at room temperature. Non-specific binding sites were blocked by 3% BSA TNT, After 3 washes with PBS-T, 2µl of sera were doted on each NC disk and left to dry. After 3 more washes, 4µl of 1:1,000 HRP-conjugated antihuman or anti-sheep IgG (Sigma, USA) were put each disk and left to dry. After washes, freshly prepared substrate solution (4-chloro-1-naphthol 340µg/ml substrate buffers with 0.03% hydrogen peroxide solution) was added to each disk. Color developed within 10-15min, wet disks were seen in day light and a well-defined blue-purple positive spot was seen by naked eye as compared with the reference control and tested sera.

Sensitivity was calculated as number of true positive divided by true positive & false negative, and specificity calculated as number of true negative divided by true positive and false negative (Attallah *et al*, 1997).

Laboratory examination: Total leukocyte counts, hemoglobin (Hb), liver function tests (AST, ALT, LDH) & cholesterol were measured using CHEM-100 Biochemistry Analyzer (Italy) and RANDOX diagnostic kits UK (Catalogue Nos HG-1539, AS-1267, Al-146, LD-487 & CH-201 respectively) after manufacturer's instructions. Packed cells volume (PCV) was measured in anti-coagulated blood samples (Henry and Davidson, 1974). Urea levels were estimated and creatinine was evaluated (Tietz, 1999).

Statistical analysis: Data were analyzed by SPSS version 23.0. M±SD deviation was calculated for quantitative variables. Student's t-test was used for comparative quantitative variables in *T. gondii* positive and negative groups with significant at $P<0.05$.

Results

Identification of specific protein fraction using EITB: Treatment of TTAg fractions on NC strips versus *T. gondii* infected women and sheep sera diluted to 1:100 (Tab 1, Fig. 1) using EITB showed eight KD protein fractions corresponding to molecular weight (M.W.) from 18 kda to 110 kda on NC strips treated by infected sera. Six from fractions

were unspecific as recorded after treatment of similar strips by non-infected control sera. Five bands corresponded to MW standard at levels of 32-34 kda, 46kda, 53 kda, 78 kda and 100 kda proved to be TTAg specific fractions as they did not react with control sera. The five groups of fractions diagnostic

sensitivity values in detecting low level of anti-*Toxoplasma* Ab in known sera was evaluated by exposing of NC strips to high dilution of same sera, which revealed that the fraction corresponding to MW of 32-34 was the most sensitive one in capturing the very low level of the anti-*Toxoplasma* Ab in sera.

Table 1: TTAg fractions reacted versus seraof infected and control humanand sheep using EITB

KDa Reacted protein fractions on NC strips treated versus			
Infected human	Non infected human	Infected sheep	Non infected sheep
110	110	100 & 120	110
100		78-80	
78	76	76	74
53	63	53	62
48	60	46-48	58-60
32		32-34	
22-26	22-26	22-26	22-26
18	18	18	18

This fraction proved 100% sensitivity in diagnosing anti-*Toxoplasma* Ab in infected women sera till 1:400 dilutions, corresponding to 0-60% sensitivity for the other fractions at dilution. Its sensitivity reached 80% in infected sheep sera corresponding to 20-50% recorded by other fractions after dilution of 20 infected \sera to 1:400. None fraction showed cross reaction with control sera. Specificity of fractions was evaluated by exposing NC strips containing the fractionated Ag to sera from human and sheep infected by other parasites. Data showed that the fraction of 32-43 kda only one did not recognize false positive reaction when treated by patient sera infected with hydatid *granulosus*, *S. mansoni* and HCV. Also, it did not react with sera of sheep infected by hydatidosis, *F. gigantica* and *C. ovies* versus control non-infected women and sheep sera.

Table 2: Sensitivity % of TTAg specific fractions versus diluted sera

Tested sera samples of	Dilution	No. +ve & Sensitivity % of different specific fractions				
		100 kda	78 kda	46 kda	53 kda	32-34 kda
Infected women (n = 5)	1:200	2/ 40%	3/ 60%	3/ 60%	5/ 100%	5/ 100%
	1:400	0.0%	0.0 %	1 / 20%	3/ 60%	5 /100%
Infected sheep (n = 20)	1:200	10/ 50%	10/ 50 %	14 / 70%	20/ !00%	20 / 100%
	1:400	4 / 20%	4 / 20%	10/ 50%	10/ 50%	16/ 80%
Vaccinated rabbit (HIRS) (n=3)	1:400	3/ 100%	3/ 100%	3/ 100%	3/ 100%	3/ 100%
	1:800	0.0%	0.0%	0.0%	1/33.3%	2/ 66.6%
	1:1000	0.0%	0.0%	0.0%	0.0%	1/ 33.3%
Non-infected women (n=20)	1:200	0.0%	0.0%	0.0%	0.0%	0.0%
Non-infected sheep (n=20)	1:200	0.0%	0.0%	0.0%	0.0%	0.0%
Non-infected rabbit (n=3)	1 :200	0.0%	0.0%	0.0%	0.0%	0.0%

32-34 kda was sensitive and specific *T. gondii* protein fraction. Fraction was 43.33% in women with history of abortion vs. to 46.66% in sheep. The 3rd & 4th cases positive in pregnant sheep were aborted and tachysoites were detected from their placenta..

Table 3: Specificity% of TTAg specific fractions versus sera of human and sheep infected by other pathogens (1:100 dilution)

Source of sera	Tested serum samples	No. +ve &specificity % of different specific fractions				
		100 kda	78 kda	46 kda	53 kda	32-34 kda
Human sera	Hydatid cyst (n=5)	1 / 80%	1/ 80%	0.0/ 100%	0.0/ 100%	0.0/ 100%
	S. mansoni (n=10)	1/ 90%	1/ 90%	1/ 90%	1/ 90%	0.0/ 100%
	HCV (n=10)	3/ 70%	3/ 70%	3/ 70%	3/ 70%	1/ 90%
Sheep sera	Hydatid (n=5)	1 / 80%	1/ 80%	0.0/ 100%	0.0/ 100%	0.0/ 100%
	F. gigantica (n=10)	3 / 70%	1/ 90%	1/ 90%	1/ 90%	0.0/ 100%
	C. ovies (n=5)	1/ 80%	1/ 80%	1/ 80%	0.0/ 100%	0.0/ 100%
Control sera	healthy women (n=20)	0.0%	0.0%	0.0%	0.0%	0.0%
	infected sheep (n=20)	0.0%	0.0%	0.0%	0.0%	0.0%

Table 4: Diagnosis of *T. gondii* infection in suspected samples by Dot ELISA

Suspected	History of samples (n = 10 in each group)	No. +ve	%
Human samples	Women with previous history of abortion	6	60%
	Pregnant women ≤ 30 years old	3	30%
	Pregnant women ≥ 30 years old	4	40%
	Total (30)	13	43.33
Sheep samples	Sheep with previous history of abortion	7	70%
	Pregnant sheep ≤ 5 years old	3	30%
	Pregnant sheep ≥ 5 years old	4	40%
	Total (30)	14	46.66

* No reaction could be recorded with sera of negative rabbit, health women and sheep of parasite free.

Table 5: Hematological and serum biochemical parameters of *T. gondii* positive and negative women .

Estimated parameters	dot ELISA +Ve suspected cases			Control cases (n=5)	
	Women with history of abortion (n=6)	Pregnant women ≤ 30 years old(n=3)	Pregnant women ≥ 30 years old(n=4)	Infected	Non-infected
Hb(g/dl)	10.5 ± 0.723b	10.7 ± 0.842b	11±0.856	11.6 ± 0.823	13.7 ± 0.720
PCV (%)	37.9±2.532	35.9±2.44	40.7± 2.323	38.7±2.449	45.2 ± 2.944
WBC/ mm3	11655 ±83 b	12395 ±77 a	11657 ± 719 b	11455 ±73 b	7398 ±509
ALP(IU/L)	88.5 ± 23.54	86.5 ± 26.54	90 ± 12.33	80.5 ± 21.64	60 ± 2.08
AST(IU/L)	33 ± 6.30 a	31 ± 5.50 a	27 ± 6.379 b	32 ± 5.30 a	11 ± 6.447
ALT(IU/L)	43± 6.033	40± 7.223	40 ± 4.656	41± 5.043	13 ± 1.231
Creatinine(mg/dl)	1.8±0.2240	1.6±0.321	1.4±0.521	1.7±0.3340	2.4 ± 1.0124
Urea (mg/dl)	46.8 ±5.75 a	43.6 ±6.66 b	42.4 ±5.33 b	44.6 ±6.75 a	22.2 ± 4.463

*Different letterers refer to significant differences at ($p \leq 0.05$)

Table 6: Hematological and serum biochemical parameters of *T. gondii* positive and negative sheep

Estimated parameters	dot ELISA +Ve suspected cases			Control cases (n=5)	
	Sheep with history of abortion(n=7)	Pregnant sheep ≤ 5 years old(n=3)	Non pregnant sheep ≥ 5 years old(n=4)	Infected	Non-infected
Haemoglobin (g/dL)	6.8±3.5	6.9±3.3	6.4±4.5	6.5±2.5	10.9±3.1
PCV (%)	26.33 ±0.123	25.11 ±0.111	23.66 ±0.213	24.41 ±0.033	35.8 ±1.01
WBC/ mm3	9830±0.122 a	9410±0.421a	9240±0.321b	9320±0.035a	5650±0.055
AST (U/L)	228± 70.340a	227± 55.231b	231± 65.431b	234.5± 90.44a	200.6±405.1
ALT (U/L)	45.344±77.4	44.765±63.4	46.224±73.4	47.33±67.4	35.62±134.8
Cholesterol (mg/dL)	231.5± 321	222.5± 331	229.5± 411	223.5± 656	195.4±63.8
LDH (U/L)	645.6±334.5	623.6±221.5	611.6±321.5	632.6±284.5	572.8±265.7
Creatinine(mg/dl)	0.9±0.324a	0.9±0.133a	1.1±0.124a	1.1±0.222a	2.1 ± 0.543
Urea (mg/dl)	34.44 ±3.4 b	33.44 ±3.2 b	36.34 ±2.4 a	38.56 ±4.55 a	26.2 ± 6.433

Hb, PCV, ALP and creatinine in infected women and sheep were non-characteristic for *T. gondii* without significant variations ($p \leq 0.05$). The deviations in WBCs, AST, and urea were significantly associated with infection than in control. Cholesterol went with previous four parameters to identify infected sheep. Acyl-CoA: cholesterol acyltransferase (ACAT) activity in *T. gondii* modulated by pharmacological ACAT inhibitors with a consequent detrimental effect on *T. gondii* replication (Sonda *et al*, 2001).

Discussion

Toxoplasma gondii is the single species of genus *Toxoplasma* (Dubey, 2008) but, it includes three strains; classified into I, II and III genetic types. Genetic type I was more virulent in mice, II in sheep and goats and might cause clinical toxoplasmosis in humans; whereas, type III was avirulent strain (Sibley, 2003). Behnke *et al.* (2011) in USA explored the genetic basis of differences in virulence between the highly virulent type I lineage and the moderately virulent type II based on successful genetic cross between these lineages. The genome-wide association revealed that a single quantitative trait locus controls the dramatic difference in lethality between these strain types. Neither ROP16 nor ROP18 previously implicated in virulence of *T. gondii* contributed to differences between types I & II. Instead, the major virulence locus contained a tandem cluster of polymorphic alleles of ROP5 that showed similar protein expression between strains. ROP5 contains a conserved serine/threonine

protein kinase domain that included only part of the catalytic triad, and hence, all the members were considered to be pseudo-kinases. Genetic disruption of the entire *ROP5* locus in the type I lineage led to complete attenuation of acute virulence, and complementation with *ROP5* restored lethality to WT levels.Small ruminants might be infected after ingestion of forage or feedstuffs polluted with sporulated oocysts or congenitally through placenta (Innes *et al*, 2009).

Fractionation of proteins using SDS-PAGE and EITB were basic techniques for identification to the specific diagnostic parasitic fraction (Rokni *et al*, 2006). The EITB assay was used as a confirmatory test for ELISA-positive sera (Hewitson *et al*, 2009). But, EITB technique was non-applicable in field settings and for large number of samples.

In the present study, reacting of fractionated TTAg on NC strips using known infected women, sheep and rabbit sera using EITB technique showed five specific protein fractions corresponding to MW of 100 kda, 78 kda, 46 kda, 53 kda & 32-34 kda. Many proteins of *T. gondii* were characterized by the Western blotting with sera from infected man or animals. Some of these antigens can induce protection when used as immunogens in mice (Letscher-Bru*et al*, 1998). Vercammen *et al*. (2000) demonstrated that some proteins from *T. gondii* antigen preparations can induce T-cell proliferation and cytokine production. Prigione *et al*. (2000) addressed cellular immunity to *T. gondii* in humans. Fatoohi *et al*. (2004) showed that in the cellular response, the most frequently recognized proteins from *T. gondii* in humans were found in the 26-37 kD range and suggested a role of bystander T cells in the induction of a humoral response to certain antigens. They added that the use of recombinant proteins to demonstrate their involvement in the immunological response would be necessary before envisaging a vaccine against *T. gondii*.

In the present study, testing the sensitivity of these fractions versus high dilutions of infected sera as well as their specificity versus sera of human and animals infected by several parasites include, hydatid cysts, *S. mansoni*, HCV, *F. gigantica* as well as *C. ovies* revealed high sensitivity and specificity for the fraction corresponding to the MW of 32-34 kda only. So, the value of this fraction provedtoxoplasmosis specific diagnostic fraction.

In the present work, smart modification of ELISA technique was applied in the form of Dot-ELISA. Swarna and Parija (2012) reported that Dot ELISA proved to be a simple, economic, direct visually read and more rapid microassay, which needed minute volume of reagents. It could be applied using specific antigenic proteins fraction separated by SDS-PAGE. It was used as an accurate test, gathering benefits of both ELISA and EITB, and applied for large number of samples (El-Bahy, 2002). No doubt, the accuracy of ELISA depended on purity and specificity degree of the used antigen and the technique could be improved when using specific purified antigenic fractions as that obtained after fractionation and immunoblot techniques (Sun *et al*, 2015).

This study developed a modification of Dot-ELISA technique using the identified specific protein fractions separated from the fractionated TTAg after EITB technique in the diagnosis of toxoplasmosis in suspected groups of women and sheep. This was followed by the opportunity of occurrence of abortion in positive cases as well as identification of some hematological and serum parameters that were associated with *T. gondii* infection to use presence of Ab in serum and deviation in specific clinical parameters as strong signal for accurate diagnosis of infection.

In the present study, the anti-*Toxoplasma* Ab in sera of suspected group of women and sheep showed the presence of specific Ab in 43.33% and 46.66% respectively. Also, the 3rd and 4th cases diagnosed as *Toxoplasma* positive in the pregnant sheep were aborted and tachysoites of *T. gondii* was detected in

their placenta.

In the present study, Dot-ELISA described used 4chloro-1-naphthol as substrate as in EITB in spite of the Orthophenyl diamine (OPD) substrate in traditional dot-ELISA procedures, which gave all the traditional benefits of dot- ELISA. Go´mez-Morales *et al*. (2012) reported that Dot-ELISA proved to be easy, directly evaluated by naked eye without the need of any special reader, and facilitated examination of large number of the samples simultaneously with same accuracy of EITB technique.

Generally speaking, congenital toxoplasmosis is well documented in Egypt. As examples, el Fakahany *et al*. (2002) in Qualyoubia Governorate found that PCR was positive in 20%, 50% and 60% of mothers with abortion, premature deliveries and deliveries of babies with congenital anomalies respectively. Said *et al*. (2011) in Cairo University Children's Hospital reported that of sixty cases, 16 (26.7%) were positive for toxoplasmosis by PCR, of which 15 (25%) had low avidity of IgG antibodies (positive). Saleh *et al.* (2014) in Alexandria General Hospital among childbearing age females reported that 22.2% pregnant women and 20% of non-pregnant ones had IHAT antibodies against *T. gondii*. On the other hand, anti-*Toxoplasma* antibodies were reported the edible animals (Rifaat *et al*, 1977), camels (Hilali *et al*, 1998), rabbits (Harfoush and Tahoon, 2010) as well as equines (Haridy *et al*, 2009), donkeys' milk (Haridy *et al*, 2010), birds (Rifaat *et al*, 1969), as well as domestic and wild rodents (Morsy *et al*, 1987). Moreover, *Toxoplasma* parasite was isolated from cats (Al-Kappany *et al*, 2010) and from stray dogs (El Behairy *et al*, 2013). Besides, *T. gondii* antibodies were reported in the Egyptian blood donors (Elsheikha *et al*, 2009).

As to fascioliasis, the estimated number of Egyptian people infection was 830,000 (Haseeb *et al*, 2002) and El Shazly *et al*. (2002) in Dakahlia Governorate reported fascioliasis in the slaughtered sheep. Rashed *et al.* (2010) stated that fascioliasis could probably come as the second endemic Egyptian hepatic helminthes infection after schistosomiasis. Ovarian cancer was high on the list of differential diagnoses; atypical clinical presentation of *Fasciola* may give rise to a misdiagnosis of malignancy (Yazici *et al*, 2005). As to hydatidosis, Haridy *et al.* (2000) reported that in the last five years, 14 cases were surgically treated in the Universities Hospitals of Cairo (9) and Ain Shams (5), and a total of 2,871,510 sheep slaughtered in the governmental abattoirs over five years (1995-1999) showed an overall hydatidosis of 0.33%. They added that sheep played the important role in disease dissemination as their cysts were the highly fertile ones as compared to other animals. Thus, the risk cycle in hydatidosis was sheep-dog-man.

As to *S. mansoni*, Elmorshedy *et al*. (2015) concluded that transmission of *S. mansoni* in high-risk areas in the Nile Delta remained uninterrupted calling for improved, more comprehensive control strategies. IL-2 & IL-4 cytokines production of cellular Th1 & Th2 immune responses respectively was associated with chronic schistosomiasis *mansoni* (stages 1-4) and chronic toxoplasmosis (El Shazly *et al*, 2005).

As to HCV, Wahib *et al.* (2006) reported that double infection with HCV and fascioliasis gave a complicated severe pathological picture and clinical course. Mohamoud *et al.* (2013) concluded that Egypt was confronted with the HCV disease burden of historical proportions that distinguished this country from others. A massive HCV epidemic at the national level must have occurred with substantial transmission still ongoing today. They added that HCV prevention must become a national priority. Policymakers, and public health and medical care stakeholders need to introduce and implement further prevention measures targeting the routes of HCV transmission Cousien *et al.* (2014) reported that data on HCV-related cirrhosis progression were scarce in developing countries in general, and in Egypt in particular.

Conclusion

The modified dot-ELISA using TTAg protein fraction of 32-34 kda proved as an easy useful tool for diagnosis of toxoplasmosis in human and animals. The technique by the method described in the present study takes the advantage of high specificity from EITB and ability to screen large number of samples from ELISA. Combination of the test with ultraion in WBCs, AST, Urea and cholesterol support the accuracy of the test results in diagnosis of toxoplasmosis on human and animal level. This would play a role in the controlstrategy to minimize the spread of the disease in the surrounding environment.

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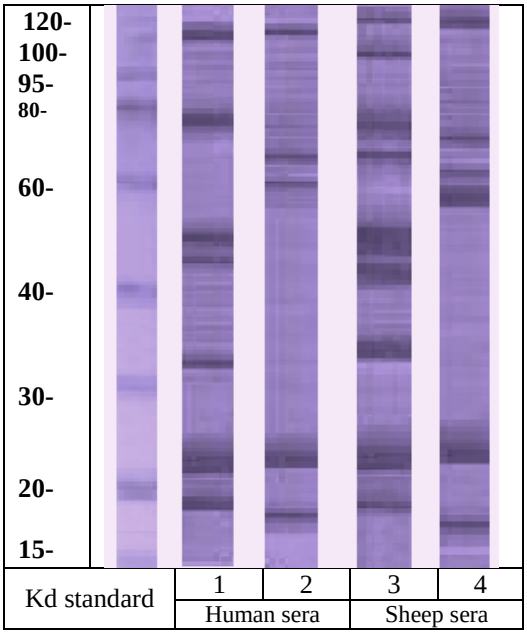

Fig. 1: Western blot profile obtained by reaction of NC strips contains fractionated *T. gondii* antigens versus infected (1) and negative (2) human sera as well as infected (3) and control (4) sheep serum samples. (Kd) is fractionated bio-Rad protein standard.