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

By
Mohamed M. El-Bahy1, Morsy Rateb Geneidy2, Lilian N. Mahrous3
And Ghada H. Abdel-Rahman4

Departments of Parasitology1,3, Faculties of Veterinary Medicine, Cairo University1,
Department of Parasitology, Faculty of Medicine, Al-Azhar University2, Nasr City,
And Beni-Sweif University3 and Department of Biology, Animal Reproduction
Research Institute4, Giza, Egypt (*Correspondence: drmelbahy@yahoo.com)

Abstract

Fractionation of Toxoplasma gondii RH strain tachyzoites (TTAg) using SDS-PAGE and iden-
tification of its specific bands versus control sera using EITB revealed five group of specific frac-
tions 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 hydaterosis, 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 con-
tents were eluted and concentrated, Dotting of 2µl from this fraction on 4mm diameter nitrocellu-
lose (NC) discs for development a modified dot-ELISA technique were evaluated in diagnosis of
Toxoplasmosis in group of suspected women and sheep. This technique succeed 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<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 ap-
plied 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 paras-
site 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 fer-
tility (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, tachy-
zoites 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 liv-
er 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 ob-
tained using a sodium dodecyl sulfate - Poly-
acylamide 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°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°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 (12x0.4cm) 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 ovis* 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°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 the eluted 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 dotted 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.

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 1: TTAG fractions reacted versus sera of infected and control human and sheep using EITB

<table>
<thead>
<tr>
<th>KDa</th>
<th>Infected human</th>
<th>Non-infected human</th>
<th>Infected sheep</th>
<th>Non-infected sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>110</td>
<td>100 &amp; 120</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>78</td>
<td>78</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>63</td>
<td>53</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>60</td>
<td>46-48</td>
<td>58-60</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>22-26</td>
<td>22-26</td>
<td>22-26</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Sensitivity % of TTAG specific fractions versus diluted sera

<table>
<thead>
<tr>
<th>Tested sera samples of</th>
<th>Dilution</th>
<th>No. +ve &amp; Sensitivity % of different specific fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected women (n=5)</td>
<td>1:200</td>
<td>2/40% 3/60% 3/60% 5/100% 5/100%</td>
</tr>
<tr>
<td>Infected sheep (n=20)</td>
<td>1:400</td>
<td>0% 0% 1/20% 3/60% 5/100% 5/100%</td>
</tr>
<tr>
<td>Vaccinated rabbit (HIRS) (n=3)</td>
<td>1:400</td>
<td>10/50% 10/50% 14/70% 20/90% 20/100%</td>
</tr>
<tr>
<td>Non-infected women (n=20)</td>
<td>1:1000</td>
<td>0% 0% 0% 0% 0%</td>
</tr>
<tr>
<td>Non-infected sheep (n=20)</td>
<td>1:200</td>
<td>0% 0% 0% 0% 0%</td>
</tr>
<tr>
<td>Non-infected rabbit (n=3)</td>
<td>1:200</td>
<td>0% 0% 0% 0% 0%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source of sera</th>
<th>Tested serum samples</th>
<th>No. +ve &amp; specificity % of different specific fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human sera</td>
<td>Hydatid cyst (n=5)</td>
<td>1/80% 1/80% 0.0/100% 0.0/100% 0.0/100%</td>
</tr>
<tr>
<td></td>
<td>S. mansoni (n=10)</td>
<td>1/90% 1/90% 1/90% 1/90% 1/90%</td>
</tr>
<tr>
<td></td>
<td>HCV (n=10)</td>
<td>3/70% 3/70% 3/70% 3/70% 1/90%</td>
</tr>
<tr>
<td>Sheep sera</td>
<td>Hydatid (n=5)</td>
<td>1/80% 1/80% 0.0/100% 0.0/100% 0.0/100%</td>
</tr>
<tr>
<td></td>
<td>F. gigantica (n=10)</td>
<td>3/70% 1/90% 1/90% 1/90% 1/90%</td>
</tr>
<tr>
<td></td>
<td>C. ovies (n=5)</td>
<td>1/80% 1/80% 1/80% 0.0/100% 0.0/100%</td>
</tr>
<tr>
<td>Control sera</td>
<td>healthy women (n=20)</td>
<td>0.0% 0.0% 0.0% 0.0% 0.0%</td>
</tr>
<tr>
<td></td>
<td>infected sheep (n=20)</td>
<td>0.0% 0.0% 0.0% 0.0% 0.0%</td>
</tr>
</tbody>
</table>

32-34 kda was sensitive and specific T. gondii protein fraction. Fraction was 43.33% in women with history of abortion vs. 46.66% in sheep. The 3rd & 4th cases positive in pregnant sheep were aborted and tachysites were detected from their placenta..
Table 4: Diagnosis of *T. gondii* infection in suspected samples by Dot ELISA

<table>
<thead>
<tr>
<th>Suspected</th>
<th>History of samples (n = 10 in each group)</th>
<th>No. +ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human samples</td>
<td>Women with previous history of abortion</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Pregnant women ≤ 30 years old</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Pregnant women ≥ 30 years old</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>Total (30)</td>
<td>13</td>
<td>43.3%</td>
</tr>
<tr>
<td>Sheep samples</td>
<td>Sheep with previous history of abortion</td>
<td>7</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>Pregnant sheep ≤ 5 years old</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Pregnant sheep ≥ 5 years old</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>Total (30)</td>
<td>14</td>
<td>46.6%</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Estimated parameters</th>
<th>Women with history of abortion (n=6)</th>
<th>Pregnant women ≤ 30 years old (n=3)</th>
<th>Pregnant women ≥ 30 years old (n=4)</th>
<th>Control cases (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>10.5 ± 0.723b</td>
<td>10.7 ± 0.842b</td>
<td>11±0.856</td>
<td>11.6 ± 0.823</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>13.7 ± 0.720</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37.9±2.532</td>
<td>35.9±2.44</td>
<td>40.7±2.323</td>
<td>38.7±2.449</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>45.2 ± 2.944</td>
</tr>
<tr>
<td>WBC/ mm3</td>
<td>11655 ±83 b</td>
<td>12395 ±77 a</td>
<td>11657 ± 719 b</td>
<td>11455 ±73 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7398 ±509</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>88.5 ± 23.54</td>
<td>86.5 ± 26.54</td>
<td>90 ± 12.33</td>
<td>80.5 ± 21.64</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>60 ± 2.08</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>33 ± 6.30 a</td>
<td>31 ± 5.50 a</td>
<td>27 ± 6.379 b</td>
<td>32 ± 5.30 a</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>11 ± 6.447</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>43±6.033</td>
<td>40±7.223</td>
<td>40±4.656</td>
<td>41±5.043</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>13 ±1.231</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.8±0.224</td>
<td>1.6±0.321</td>
<td>1.4±0.521</td>
<td>1.7±0.340</td>
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<tr>
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<td></td>
<td>2.4 ± 1.024</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>46.8±7.55 a</td>
<td>43.6±6.66 b</td>
<td>42.4±5.33 b</td>
<td>44.6±6.75 a</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>22.2 ± 4.463</td>
</tr>
</tbody>
</table>

* Different letterers refer to significant differences at (p≤0.05)

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

<table>
<thead>
<tr>
<th>Estimated parameters</th>
<th>dot ELISA + Ve suspected cases</th>
<th>Control cases (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>Sheep with history of abortion (n=7)</td>
<td>Pregnant sheep ≤ 5 years old (n=3)</td>
</tr>
<tr>
<td></td>
<td>6.8±3.5</td>
<td>6.9±3.3</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>26.3±0.123</td>
<td>25.11±0.111</td>
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<tr>
<td>WBC/ mm3</td>
<td>98.9±0.122a</td>
<td>94.1±0.421a</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>AST (U/L)</td>
<td>228±7.0.340a</td>
<td>227±5.231b</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>45.34±7.4</td>
<td>44.765±63.4</td>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>231.5±321</td>
<td>227.5±331</td>
</tr>
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<tr>
<td>LDH (U/L)</td>
<td>645.6±334.5</td>
<td>623.6±221.5</td>
</tr>
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</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9±0.324a</td>
<td>0.9±0.133a</td>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>34.44±3.4b</td>
<td>33.44±3.2b</td>
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</tbody>
</table>

Hb, PCV, ALP and creatinine in infected women and sheep were non-characteristic for *T. gondii* without significant variations (p≤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 acyl-transferase (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-Bruet 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 proved toxoplasmosis 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 immuno-blot 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 tachyzoites of T. gondii was detected in...
their placenta.

In the present study, Dot-ELISA described used 4-chloro-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 Qualyoubi 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 (Hardy et al., 2009), donkeys’ milk (Hardy 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 (El-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 cho-

References
Al-Kappany, YM, Rajendran, C, Abu-El-
wafa, SA, Hilali, M, Su, C, 2010: Genetic diver-
sity of Toxoplasma gondii isolates in Egyptian
feral cats reveals new genotypes. J. Parasitol. 96,
6:1112-4.
AttallahAM, el-Masry SA, Rizk H, Ismail H,
using urine, a rapid and dependable field assay
for diagnosis of schistosomiasis. J. Egypt. Soc.
Parasitol. 27, 1: 279-89.
Behnke, MS, Khan, A, Wootton, JC, Dubey, J
Toxoplasma mediated by amplification of a fam-
Cocuz, ME, 2014: Diagnostic Difficulties in
Human Trichinellosis: A case report. Bull. Trans-
vilania University of Brașov Series VI: Med.
Sci. 7, 56:2-9
Cousien, A, Obach, D, Deuffic-Burban, S,
ion reliable when estimating transition proba-
bilities? the case of HCV-related cirrhosis in
Dubey, JP, 2008: The history of Toxoplasma
gondii-the first 100 years. J. Eukaryotic Micro-
biol. 55:467-75.
Edward, JF, Dubey, JP, 2013: Toxoplasma go-
ndii abortion storm in sheep on a Texas farm and
isolation of mouse virulent atypical genotype T.
gondii from an aborted lamb from a chronically
El-Fakahany, AF, Abdel-Maboud, AI, el-Gar-
hy, MF, Eraky, MA, 2002: Comparative study
between ELISA IgG, IgM & PCR in diagnosing
and studying toxoplasmosis in Qualyobia Gove-
norate, Egypt. J. Egypt. Soc. Parasitol. 32, 2:
475-86.
El Behairy, AM, Choudhary, S, Ferreira, LR,
Kwok, OC, Hilali, M, Su, C, et al, 2013: Gen-
etic characterization of viable Toxoplasma go-
dii isolates from stray dogs from Giza, Egypt.
El-Bahnasawy, MM, Ahmed, GMS, Gaber, W
AI, Morsy, TA, 2013: The infantile visceral le-
ishmaniasis: Could it attack Egyptian North Co-
stal Region Again? J. Egypt. Soc. Parasitol. 43,
El-Bahnasawy, MM, Dabbous, HKh, Morsy,
TA, 2010: Imported malaria as a threat to Egypt.
El-Bahy, NM, 2002: Diagnosis of Fasciola in-
festation by Dot ELISA technique using SDS-
PAGE eluted excretory secretary (ES) protein
fractions. 2nd Veterinary Cong. (23-24 April):
Elmorsheidy, H, Bergquist, R, El-Ela, NE,
schistosomiasis mansoni control be sustained in
high-risk transmission foci in Egypt? Parasit.
Vectors Jun 16:8.372. doi: 10.1186/s13071-015-
0983-2.
El-Shazly, AM, el-Wafa, SA, Haridy, FM, So-
liman, M, Morsy TA, et al, 2002: Fascioliasis
among live and slaughtered animals in nine cen-
Parasitol. 32, 1:47-57.
El-Shazly, AM, Azab, MS, El-Beshbishi, SN,
El-Nahas, HA, Abdel Magied, AA, et al, 2005:
Some molecular aspects in schistosomiasis manso-
35, 3:795-808.
Elshiekh, HM, El-Motayam, MH, Abouel-
Nour, F, Morsy, AT, 2009: Oxidative stress and
immune-suppression in Toxoplasma gondii posi-
tive blood donors: implications for safe blo-
od transfusion. J. Egypt. Soc. Parasitol. 39, 2:
421-8
Fatoohi, AF, Cozon, GJ, Gonzalo, P, Mayen-
con, M, Greenland, T, et al, 2004: Heteroge-
neity in cellular and humoral immune responses
against Toxoplasma gondii antigen in humans.
Gasbarre, LC, Suter, P, Fayer, R, 1984: Hum-
eral and cellular immune responses in cattle and


nomic and early diagnosis among Egyptian pre-
Saleh, AMA, Ali, HA, Ahmed, SAM, Hosny, S M,
Morsy, TA, 2014: Screening of Toxoplasma
gondii infection among childbearing age females
and assessment of nurses’ role in prevention and
44, 2:329-34.
Shaheen, HI, Kamal, KA, Farid, Z, Mansour,
N, Doctor, FN, et al, 1989: Dot-ELISA for the
75:549-52.
Sibley D, 2003: Recent origins among ancient
Sonda, S, Ting, LM, Novak, S, Kim, K, Mah-
er, JJ, et al, 2001: Cholesterol esterification by
host and parasite is essential for optimal prolif-
eration of Toxoplasma gondii. J. Biol. Chem.
276, 37:34434-40.
Sun, GG, Wang, ZQ, Liu, CY, 2015: Early
serodiagnosis of trichinellosis by ELISA using
excretory–secretory antigens of Trichinella spi-
Swarna, SR, Parija, SC, 2012: Evaluation of
Dot-ELISA and enzyme-linked immuno-electro-
transfer blot assays for detection of a urinary
hydatid antigen in the diagnosis of cystic echino-
Tietz, NW, 1999: Textbook of Clinical Chemis-
try. (3rd ed.) Burris, CA, and Ashwood, ER, Saun-
ders.
Towbin, H, Stachelin, T, Gordon, J, 1979:
Electrophoretic transfer of proteins from poly-
acrylamide gels to nitrocellulose sheets: Proce-
dures and some applications. Proc. Nat. Acad.
Sci. USA, 76:4350-4.
Tsai, CM, Frasch, CE, 1982: A sensitive silver
stain for detecting lipopolysaccharides in poly-
Vercammen, M, Scorza, A, Huygen, K, De-
Braekeleer, J, Diet, R, et al, 2000: DNA vacc-
ination with gene encoding Toxoplasma gondii
antigens GRA1, GRA7 and Rop2 induce partially
protective immunity against lethal challenge
Wahib, AA, Seif El Nasr, MS, Mangoud, A
M, El Shazly, AM, Morsy, ATA, 2006: The
clinical picture of hepatitis C virus as a concomi-
ant infection with fascioliasis. J. Egypt. Soc.
Parasitol. 36, 1:51-62.
Wishahy, AO, Rifaat, MA, Morsy, TA, El-Na-
gger, BA, 1972: Toxoplasmosis in children with
Hyg. 75, 12:255-6.
Yazici, G, Dilek, U, Karahacak, T, Ertunc, D,
Korkmaz, M, 2005: Adnexal fascioliasis masq-
erading as ovarian cancer. Gynecol. Oncol.

![Western blot profile](image)

**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.