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Research Article

# Diagnosis of Human Toxoplasmosis Using *Toxoplasma gondii* Tachyzoites Crude Antigen of Different Origin

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## Abstract

In the present study, crude extract of *Toxoplasma gondii* tachyzoites were prepared from chicken local isolate and adopted for diagnosis of chicken toxoplasmosis by ELISA. The extract proved success in detection of Toxoplasma antibodies in 275 (78.12%) out of 352 examined free rang chicken serum samples. The important facet of the present study is adoption of crude chicken tachyzoites antigen in diagnosis of human toxoplasmosis, where it proved 100% sensitivity in the detection of Toxoplasma antibodies in all examined cases (61 samples) of human toxoplasmosis. Meanwhile, RH strain antigen, which used to be utilized in diagnosis of human toxoplasmosis, detected only 80% of chicken toxoplasmosis samples. By SDS-PAGE, chicken tachyzoites antigen resolved to 8 bands of molecular weights ranged from 30-116 kDa. Also, RH strain antigen revealed 10 bands with different molecular weights ranged from 23-166 kDa. The immunoreactive bands were detected by immunoblotting assay. One band of 116 kDa was detected in chicken antigen and 28 kDa in RH antigen by infected human sera. While, two bands with molecular weights 116 and 83 kDa and 116 and 30 kDa were detected in chicken tachyzoites and RH antigens, respectively using infected chicken sera. A band of 116 kDa was detected in chicken antigen using both sera, which is mainly responsible for diagnosis of chicken and human toxoplasmosis. In conclusion, chicken tachyzoites antigen based ELISA can be successfully utilized in diagnosis of chicken and human toxoplasmosis.

Key words: *Toxoplasma gondii,* chicken, human, toxoplasmosis, immunoblot, SDS-PAGE

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Toxoplasma gondii* is a successful food and water borne parasite that infects approximately 1 billion people worldwide (Xiao and Yolken, 2015). Also, it has been considered as one of the most successful eukaryotic pathogens concerning the number of host species infected globally (Dubey, 2010a, b; Zhao *et al*., 2011). In Egypt few studies were focused on the role and importance of free rang chicken (*Gallus domesticus*) in transmission of toxoplasmosis to human. Chicken meat considered as important part of kitchen, where it consumed widely around the world. Therefore, consumption of uncooked or not properly cooked poultry meat may pose a risk factor for *T. gondii* infection of human and animals (Dubey, 2010b).

Chicken considered as important intermediate host of *Toxoplasma gondii* and it can shed millions of infective resistance oocyst on the ground surrounding human household. Free rang chicken feeding on the ground so the prevalence of *T. gondii* in chicken is a good indicator of soil contamination with *T. gondii* oocysts (El-Massry *et al*., 2000; Dubey and Jones, 2008). Examination of free-range chicken in Egypt using the Modified Agglutination Test (MAT) showed a sero-prevalence of *T. gondii* antibodies of 47.2% (El-Massry *et al*., 2000). In addition, investigation using an indirect hemagglutination test (IHAT) showed a lower percentage (38.1%) of seropositive chicken (Harfoush and Tahoon, 2010). While, (Barakat *et al*., 2012) recorded high prevalence of chicken toxoplasmosis (69.5%) using ELISA. For other countries, using MAT, *T. gondii* antibodies were detectable in 44.4% in South America (Dubey *et al*., 2005a), 52% in West Indies (Dubey *et al*., 2005b) 64% in Ghana, 24.4% in Indonesia, 12.5% in Italy, 30% in Poland and 24.2% in Vietnam (Dubey *et al*., 2008).

The used antigen in serological diagnosis of human toxoplasmosis depends mainly on RH antigen which is of mice origin. There are previous trials on isolation of tachyzoites from infected mice (Beghetto *et al*., 2006) and horses (Hassan *et al*., 2012) and used it successfully in diagnosis of human toxoplasmosis. Consequently, the aims of the present study is to isolation of *T. gondii* tachyzoites from free rang chicken and assess its potency in diagnosis of human and chicken toxoplasmosis.

## MATERIALS AND METHODS

**Isolation and maintenance of chicken *T. gondii* tachyzoites:** Chicken pooled tissue samples, about 25-50 g from heart, brain and pectoral muscles were prepared as described by Shaapan and Ghazy (2007). The tissues and organs were cut into small pieces and stored at 4°C for few hours until used for bioassay in mice. The isolation of *T. gondii* local strain was carried out according to Sharma and Dubey (1981) followed by microscopically examination for the presence of *T. gondii* tissue cysts containing the bradyzoites. Bioassay of the digested positive tissues in mice was carried according to Dubey (2010a), in order to obtain the tachyzoites stage. Chicken tachyzoites were maintained through repeated intra peritoneal mouse inoculation in mice in the Zoonotic Diseases Department, National Research Center according to the procedures of Johnson *et al*. (1979).

**Isolation of *T. gondii* RH strain:** Virulent RH strain of *T. gondii* was obtained from colony maintained in Department of Zoonosis, National Research Center by serial passage in mice according to the method of Johnson *et al*. (1979).

**Antigens preparation:** The RH antigen and chicken local strain antigen of *T. gondii* achyzoites were prepared as described by Waltman *et al*. (1984). Briefly, tachyzoites were repeatedly freeze and thawed to rupture the parasite wall, sonicated and centrifuged at 12,000 rpm for 30 min at 4°C. The supernatants were separately collected and its protein content was determined by the method of Lowry *et al*. (1951).

### Collection of serum samples

**Chicken sera:** About 352 chicken serum samples were collected from different regions in Egypt. Forty samples out of them which proved to be strongly positive by ELISA were selected and designated. Serum samples were labeled in serial numbers and stored at -20°C until use.

**Human sera:** A total of 73 human serum samples (61 positive and 12 negative), which were previously examined by ELISA using two different Toxoplasma antigens and commercially ELISA Kit by Hassan *et al*. (2012). Samples were labeled in serial numbers and stored at -20°C until use.

### Characterization of chicken Toxoplasma antigen

**SDS-PAGE:** Chicken antigen was characterized by SDS-PAGE compared with RH antigen according to the procedures described by Laemmli (1970). Gel was stained with silver stain according to Wray *et al*. (1981).

**Immunoblot assay:** Immunoblot assay was utilized to identify immunoreactive bands in chicken and RH *T. gondii*

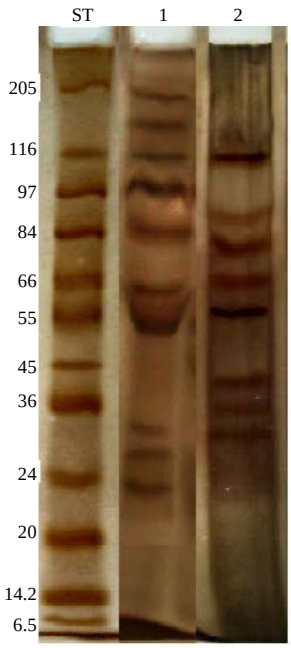


Fig. 1: SDS-PAGE, Line 1: RH antigen, Lane 2: Chicken antigen and Line St: Molecular weight standards in kDa

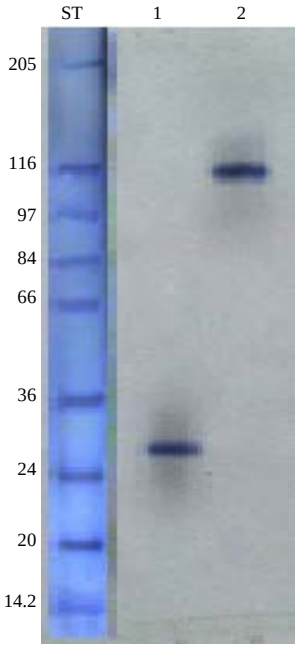


Fig. 2: Western blot, Line 1: Reactivity of infected human sera antibodies with RH antigen, Lane 2: Chicken antigen and Line St: Molecular weight standards in kDa

antigens using infected human and chicken sera. The assay was carried out as the method described by Towbin *et al.* (1979).

**Enzyme linked immunosorbent assay:** The ELISA was used for evaluation the diagnostic potential of chicken antigen against chicken and human sera. Also it was used to detect efficacy of RH antigen in detection of chicken Toxoplasma antibodies in infected chicken sera. The optimum antigen concentration, serum and conjugate dilutions were determined by checkerboard titration (Shaapan *et al.*, 2008) and test procedures were carried out according to the method described by Lind *et al.* (1997).

**Ethics statement:** The study is approved ethically by the Medical Research Ethics Committee-National Research Centre, El Bohouth St., Dokki, Giza, Egypt.

## RESULTS

**Electrophoretic profile of chicken and RH antigens of *T. gondii* tachyzoites:** The electrophoretic profile of RH antigen revealed 10 bands of molecular weights ranged from 23-166 kDa (Fig. 1) in lane 1. While, chicken antigen showed 8 bands with different molecular weights ranged from 30-116 kDa (Fig. 1) in lane 2.

**Immunogenic bands:** Using infected human sera one immunoreactive band of molecular weight 28 kDa was detected in RH antigen (Fig. 2) in lane 1 and a band of 116 kDa was detected in chicken antigen (Fig. 2) in lane 2. Also, infected chicken sera identified two bands of 116 and 30 kDa in RH antigen (Fig. 3) in lane 1 and 116 and 83 kDa in chicken antigen (Fig. 3) in lane 2.

**Potency of chicken antigen in diagnosis of chicken and human toxoplasmosis:** Figure 4 displayed the using of chicken *T. gondii* isolate as a diagnostic antigen in detection of chicken toxoplasmosis indicating that 275 out of 352 examined serum samples were positive and proved prevalence of 78.12%. Also, Fig. 5 showed the success of chicken antigen in discrimination between seropositive and seronegative of previously examined human serum samples.

**Evaluation of RH antigen in detection of Toxoplasma antibodies in infected chicken sera:** The RH antigen can detect Toxoplasma antibodies in only 32 out of 40 (80%) Toxoplasma chicken infected serum samples, which previously proved to be highly positive by ELISA (Fig. 6).

## DISCUSSION

Sero-diagnosis based ELISA was previously described as a promising method for detection of toxoplasmosis in human and animals. In the present study, the applicability of the same procedure for sero-diagnosis of toxoplasmosis in naturally infected free-range chicken and human was evaluated. There is no commercially kit for diagnosis of toxoplasmosis in chicken. In addition, most commercial assays for detecting specific anti human-Toxoplasma immunoglobulins use RH strain of *T. gondii* tachyzoites antigen. In the present study, crude chicken antigen of *T. gondii* was used and proved that 275 (78.12%) out of 352 examined chicken serum samples were infected with *T. gondii*. This high prevalence refers to importance of chicken meat as a source of infection to Egyptian consumers.

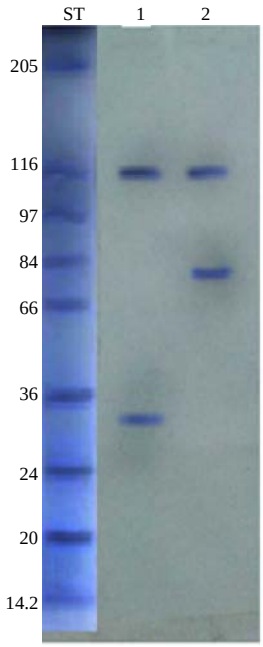


Fig. 3: Western blots, Line 1: Reactivity of infected chicken sera antibodies with RH antigen, Lane 2: Chicken antigen and Line St: Molecular weight standards in kDa

Results of the present study confirm earlier observation detected by Barakat *et al.* (2012), where they detected 69.5% free range chicken infected with *T. gondii* in Egypt by ELISA. But less prevalence rates were detected; 47.2% (El-Massry *et al.*, 2000), 40.4% (Dubey *et al.*, 2003) using MAT technique and 38.1% by Harfoush and Tahoon (2010) by IHAT. This difference in infection percentages may be due to high sensitivity of ELISA than other assays in addition to utilized antigen in the present study.

High costs of importing commercial ELISA kits and difficulty of isolation of tachyzoites from women placenta and aborted fetus directed our attention to prepare antigen from tachyzoites isolated from other origin and used it in diagnosis of human toxoplasmosis. In the present study, chicken crude tachyzoites antigen was successfully utilized in the detection of human toxoplasmosis, where it can discriminate between seropositive and seronegative human serum samples, which previously examined by two different Toxoplasma antigens

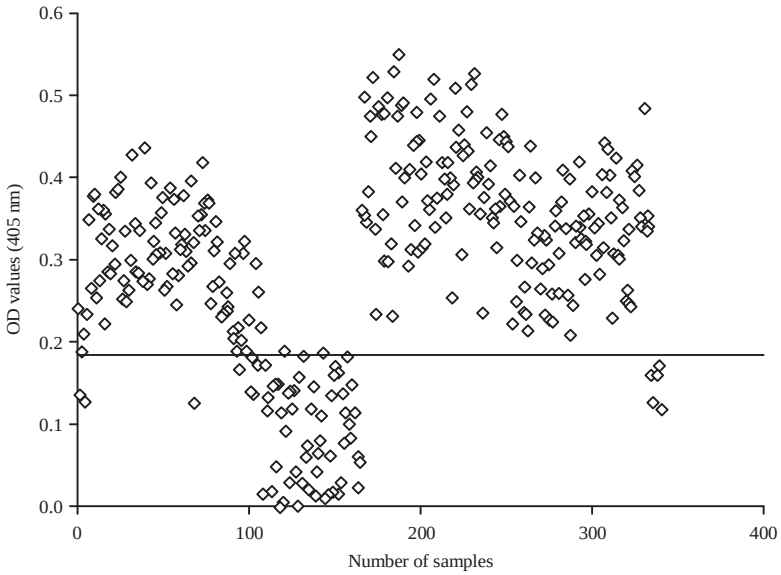


Fig. 4: Scatter-graph represents the potency of chicken antigen in diagnosis of chicken toxoplasmosis horizontal line represents cut off value

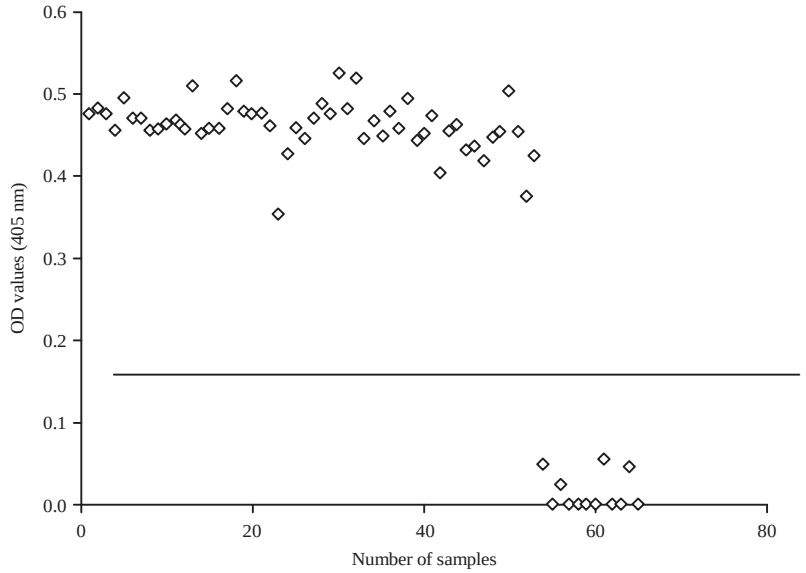


Fig. 5: Scatter-graph represents the potency of chicken antigen in detection of Toxoplasma antibodies in human sera. Horizontal line represents cut off value

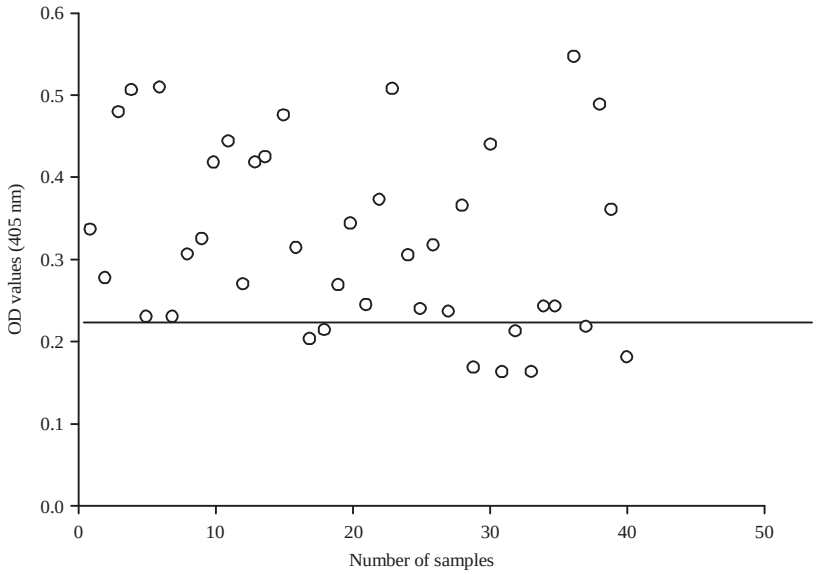


Fig. 6: Scatter-graph represents the potency of RH antigen in detection of Toxoplasma antibodies in infected chicken sera. Horizontal line represents cut off value

and commercial ELISA kit by Hassan *et al.* (2012). Results of the present study confirm our earlier observations in our laboratory, where horse derived antigen was successfully utilized in diagnosis of human toxoplasmosis (Hassan *et al.*, 2012) and proved 100% sensitivity. Moreover, Toaleb *et al.* (2013) succeeded in utilization of Toxoplasma antigen from camel origin in diagnosis of cattle toxoplasmosis.

Chicken tachyzoites antigen was characterized by SDS-PAGE and revealed 8 bands of molecular weights ranged from 30-116 kDa. To this knowledge no literatures were available concerning characterization of chicken tachyzoites antigen by SDS-PAGE, but a comparable band of molecular weight 115 kDa were isolated from *T. gondii* antigen of horse origin (Ghazy *et al.*, 2007).

In the present study RH antigen was resolved into 10 bands with molecular weights ranged from 23-166 kDa. A comparable number of bands; 11 of RH antigens were previously introduced by Hassan *et al.* (2012). While,

Hassanain *et al*. (2013) showed that RH antigen revealed 17 bands. This difference between this results and the previous ones in the number of bands may be due to difference in the extraction procedures or difference in preparation of antigen before loading to the gel.

In present study one immunoreactive band of molecular weight 116 kDa in chicken tachyzoites antigen and one band of molecular weight 28 kDa in RH antigen were detected by immunoblott assay using infected human sera. While, two bands of molecular weights 116 and 83 kDa and 116 and 30 kDa were detected in chicken and RH antigens, respectively using infected chicken sera. A band of molecular weight 116 kDa was detected in chicken tachyzoites by using either infected human sera or infected chicken sera. This means that 116 kDa was responsible for the success of chicken antigen in diagnosis of chicken and human toxoplasmosis. In previous study a band of molecular weight 116 kDa was previously detected in crude and purified fraction of RH antigen and successfully utilized in diagnosis of mice toxoplasmosis (Hassanain *et al*., 2013).

## CONCLUSION

The present study introduced crude chicken tachyzoites antigen which can be successfully utilized in diagnosis of human and chicken toxoplasmosis. A band of 116 kDa that was identified in both antigens using chicken infected sera and identified in chicken antigen by both sera needs to be isolated in future study. It can be helpful in control and treatment of this important disease.

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