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Research Article
Diagnosis of Toxoplasmosis Using Affinity Purified Fraction of Tachyzoites Local Isolate

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Abstract

To increase diagnostic potency of antigen, its immunogenic component must be isolated. Immuno-affinity column chromatography was adopted to purify immunogenic fraction of *Toxoplasma gondii* tachyzoites of chicken local isolate. The isolated fraction was characterized by SDS-PAGE, where it exhibited 2 bands of molecular weights 116 and 83 kDa compared with 8 bands of molecular weights ranged from 116-30 kDa in crude antigen. The isolated fraction proved high diagnostic activity compared with crude antigen by ELISA using two fold serial dilutions of infected human and chicken sera. The fraction detected antibodies in 366 out of 406 (90.1%) examined free range chicken serum samples. Also, it proved success in the detection of Toxoplasma antibodies in 30 serum samples of patient infected with *T. gondii*. The fraction proved 100% sensitivity and 84.4% specificity. Its Positive Predictive Value (PPV), Negative Predictive Value (NPV) and diagnostic accuracy were 85.7, 100 and 91.9%, respectively. In conclusion, the present study introduced novel *T. gondii* chicken tachyzoites partially purified fraction proved high diagnostic activity of chicken and human toxoplasmosis.

Key words: *Toxoplasma gondii,* chicken and human toxoplasmosis, affinity purified fraction, SDS-PAGE

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

INTRODUCTION

Toxoplasmosis caused by *Toxoplasma gondii* is one of the most common parasitic infections of humans and other warm blooded animals. The cost of illness in the US caused by Toxoplasma has been estimated to be nearly US $3 billion annually (Batz *et al*., 2012; Hoffmann *et al*., 2012). Recent publications have linked suicide and schizophrenia to Toxoplasma infection (Torrey *et al*., 2012). Moreover, Mead *et al*. (1999) suggested that *T. gondii* is one of three pathogens (together with Salmonella and Listeria), which account for >75% of all deaths due to food borne disease in the USA.

Free rang-chicken is one of the major food sources for human. Therefore, it can be considered as a potential source of human toxoplasmosis (Wang *et al*., 2015). The rate of chicken toxoplasmosis was ranged from 30-50% (Zhu *et al*., 2008; Zhao *et al*., 2012), 78.12% in free rang chicken (Hassan *et al*., 2015) and reached to 100% in backyard chickens (Dubey, 2010a; Dubey *et al*., 2010). But chickens always display chronic infection without apparent clinical signs to toxoplasmosis (Zou *et al*., 2011). In addition, complicating matters is the fact that the number of *T. gondii* organisms in meat from naturally infected food animals are very low, making the parasite difficult and expensive to detect by direct methods. It is estimated that as few as one tissue cyst may be present in 100 g of meat (Dubey, 2010b). Moreover, there is no predilection site for Toxoplasma in meat animals, virtually all edible portions of an animal can harbor viable *T. gondii* tissue cysts and tissue cysts can remain viable in food animals for years (Dubey *et al*., 1986).

So, serological diagnosis considered as precise method for toxoplasmosis. On the other hand, infected chicken with toxoplasmosis play an important role in epidemiology of human toxoplasmosis (Dubey *et al*., 2004; Aboelhadid *et al*., 2013). Consequently, the aim of this study was to isolate immunodiagnostic fraction of chicken isolate *Toxoplasma gondii* tachyzoites antigen using affinity column chromatography and using it in diagnosis of chicken and human toxoplasmosis.

MATERIALS AND METHODS

***Toxoplasma gondii* chicken local strain:** The strain was isolated from pooled tissues of heart, brain and pectoral muscles of slaughtered chicken at different regions in Egypt. The meat and tissue samples were digested using pepsin digestive solution as described by Sharma and Dubey (1981). Virulent local strain of *T. gondii* was obtained by bioassay of suspected infected chicken tissues in mice according the method of Dubey *et al*. (2010) and the obtained isolate was confirmed as *T. gondii* by polymerase chain reaction.

Antigen preparation: Soluble crude antigen was prepared from chicken tachyzoites local isolate according to Waltman *et al*. (1984), briefly, tachyzoites were repeatedly freezing and thawing to rupture the parasite wall, sonicated and centrifuged at 12,000 rpm for 45 min at 4°C. The supernatant was collected and its protein content was determined by the method of Lowry *et al*. (1951) and antigen was aliquoted and stored at -20°C until use.

Samples collection

Chicken serum samples: Four hundred and six random chicken blood samples were collected from different localities in Egypt. Also, positive blood samples, which proved to be strongly positive by ELISA were selected and designated. Serum samples were obtained by centrifugation of coagulated blood, labeled in serial numbers and stored at -20°C until use.

Human serum samples: Sixty two human blood samples were investigated serologically by ELISA for detection of *T. gondii* IgG antibodies and parasitologically for other parasitic infections. According to these examinations, human sera were classified into 3 groups: Group (I) included 30 patients were positive for *T. gondii* IgG serologically and negative for other parasitic infections parasitologically. Group (II) included 21 patients were negative for toxoplasmosis and positive for other parasitic infections; 10 schistosomiasis *mansoni*, 5 fascioliasis, 3 amoebiasis and 3 hydatidosis. Group (III) included 11 individuals negative for toxoplasmosis and other parasitic infections used as control negative.

Purification of chicken *T. gondii* tachyzoites local strain antigen: Sepharose 4B affinity column chromatography was adopted for purification of chicken tachyzoites crude antigen. In brief, chicken *T. gondii* positive sera were dialyzed for three days against 0.1 M $NaHCO_3$ buffer pH 8.3 containing 0.5 M NaCl and 0.02% NaN_3 and coupled to cyanogen bromide-sepharose 4B (CNBr-Sepharose 4B) swollen beads (Sigma-Aldrich, USA) by strictly using the manufacturer instructions. Bound fraction was eluted with 50 mM glycine, pH 2.3 containing 500 mM NaCl pH 2.3 (Ahn *et al*., 1997). Protein concentrations of unbound and purified antigens were assayed by the method of Lowry *et al*. (1951).

SDS-PAGE: Crude chicken *T. gondii* tachyzoites antigen and its isolated fraction were separately mixed with sample buffer containing 2-mercaptoethanol before loading to the slab gel using 10% SDS-PAGE (Laemmli, 1970). After separation, gel was fixed in 50% methanol and stained with silver stain according to Wray *et al.* (1981). Relative molecular weights of bands were calculated using molecular weight marker.

ELISA: The diagnostic potency of crude antigen and the isolated fraction was assessed by indirect ELISA and two fold serial dilutions of infected chicken and human sera. The most potent antigen was adopted to detect toxoplasmosis infection in collected chicken and human serum samples. Antigen specificity was assessed against human infected serum samples with schistosomiasis *mansoni*, fascioliasis, amoebiasis and hydatidosis. The ELISA was performed, antigens concentrations, sera and conjugate dilutions were determined by checkerboard titration (Santiago and Hillyer, 1988) and the cut off value was calculated were determined according method of Shaapan *et al.* (2008).

Statistics: Statistical analysis was performed with unpaired student's t-test using computer software package GraphPad Prism 5.02 version to compare groups. Sensitivity, specificity, diagnostic accuracy, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of isolated fraction were calculated according to Gonzalez-Sapienza *et al.* (2000).

RESULTS

Electrophoretic profile of antigens: The isolated fraction was resolved into two bands of 116 and 83 kDa in lane 2 (Fig. 1) compared with 8 bands associated with crude extract (116, 87, 83, 66, 55, 40, 36 and 30 kDa in lane 1 (Fig. 1).

Diagnostic potency of crude extract and isolated fraction: Isolated fraction proved higher diagnostic potential compared with crude extract in detection of chicken and human toxoplasmosis (Fig. 2a and b), respectively. Activity of isolated fraction was still potent, where it detected Toxoplasma IgG at dilution (1:4096).

Diagnostic potential of isolated fraction: Based on checker board titration results for ELISA, the optimum concentration of antigen was 20 µg mL^{-1}, while the dilution of antibodies was 1:100 and the dilution of conjugate was 1:1000. The isolated fraction can detect Toxoplasma antibodies in 366 out

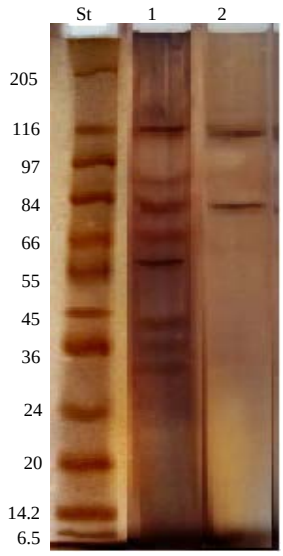

Fig. 1: Electrophoretic profile of the isolated fraction (lane 2) compared with crude extract of *T. gondii* chicken isolate (lane 1). Molecular weight standards in kDa (Lane St)

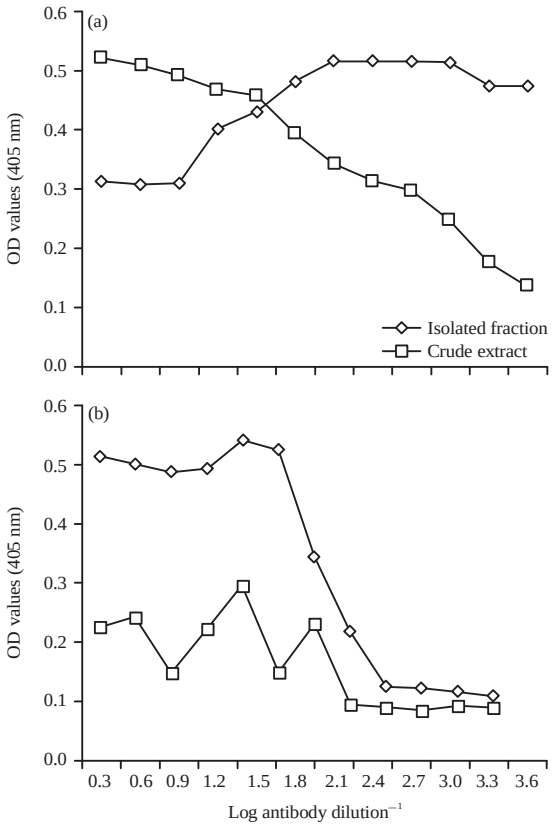

Fig. 2(a-b): Diagnostic potentials of isolated fraction and crude extract of *T. gondii* chicken tachyzoites local isolate against infected (a) Chicken sera and (b) Human sera

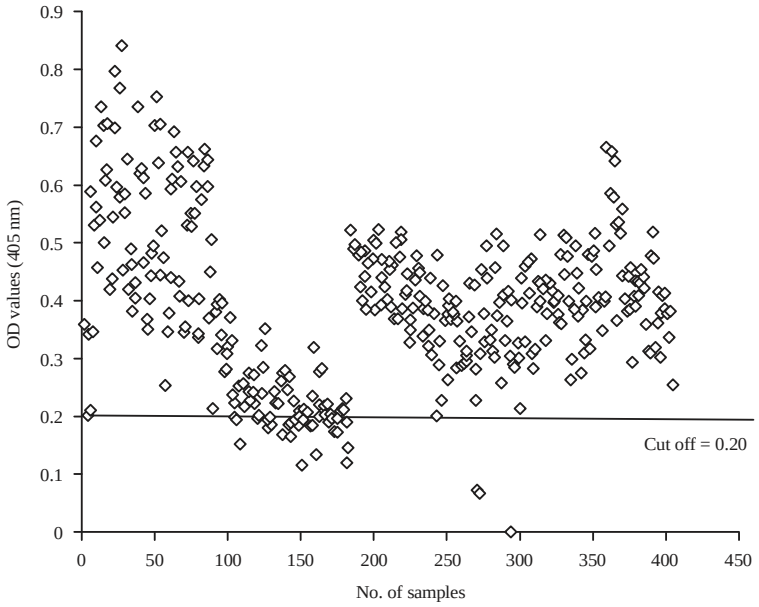

Fig. 3: Diagnostic potentials of affinity isolated fraction of *T. gondii* chicken local isolate in diagnosis of chicken toxoplasmosis

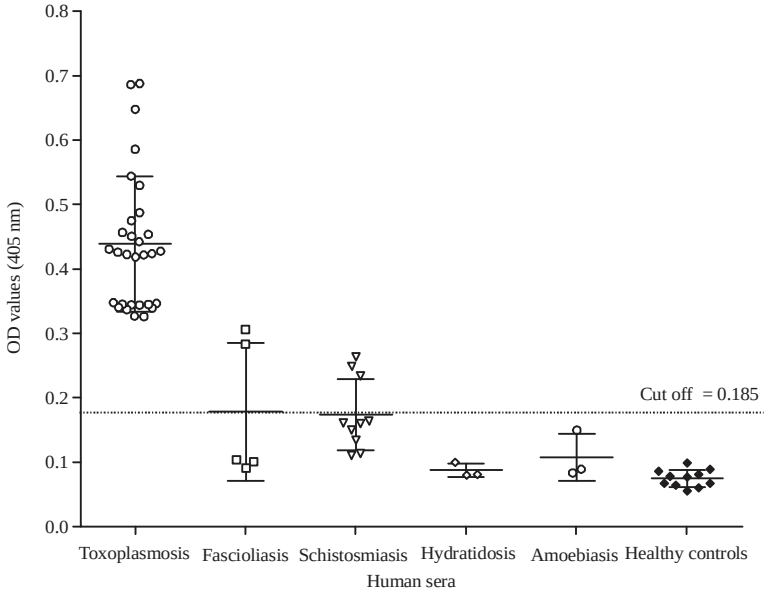

Fig. 4: Diagnostic activity of affinity isolated fraction of *T. gondii* chicken local isolate in diagnosis of human toxoplasmosis and other parasitic infections

Table 1: Sensitivity, specificity, PPV, NPV and diagnostic accuracy percentages of isolated fraction against infected human sera by ELISA

Antigen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Isolated fraction	100	84.4	85.7	100	91.9

ELISA: Enzyme linked immunosorbent assay, PPV: Positive predictive value and NPV: Negative predictive value

of 406 (90.1%) of examined chicken serum samples (Fig. 3). Also, it was succeeded in detection of toxoplasmosis in 30 patient sera infected with Toxoplasma. Isolated fraction was cross-reacted with 2 out of 5 patient infected with fascioliasis and 3 out of 10 human schistosomiasis. While, it did not react with human sera infected with Amoeba and hydatid cyst. Fraction recorded 100% sensitivity and 84.4% specificity (Fig. 4 and Table 1). Also, PPV%, NPV% and diagnostic accuracy percentage were 85.7, 100 and 91.9%, respectively (Table 1).

DISCUSSION

Due to significant medical and veterinary problems caused by *T. gondii*, the present study focused on accurate diagnosis of toxoplasmosis. Accurate diagnosis of the disease is considered a key to resolve the problem. So, utilization of specific antigen and a sensitive method is essential. In previous study in this laboratory, purified fraction of *T. gondii* horse derived antigen proved high efficacy in diagnosis of human toxoplasmosis (Hassan *et al*., 2012). Moreover, affinity purified fraction of *T. gondii* camel isolate was successfully utilized in diagnosis of cattle toxoplasmosis (Toaleb *et al*., 2013). Recently, chicken *T. gondii* crude tachyzoites antigen proved success in diagnosis of chicken and human toxoplasmosis (Hassan *et al*., 2015). This last result in addition to previous ones directed this attention to isolate the components, which responsible for this success. So, CNBr-Sepharose 4B affinity column chromatography was adopted for purification of chicken tachyzoites crude antigen using naturally infected chicken sera.

The purified fraction proved higher diagnostic potency compared with crude antigen in detection of Toxoplasma antibodies in two fold serially diluted chicken and human sera. The fraction resolved into two bands of molecular weight 116 and 83 kDa by SDS-PAGE. This result confirms our earlier observation that two bands of 116 and 83 kDa were detected in crude chicken *T. gondii* tachyzoites antigen by immunoblot assay using infected chicken serum (Hassan *et al*., 2015). A single band of molecular weight 116 kDa was previously detected in affinity purified fraction of RH antigen and successfully utilized in diagnosis of mice toxoplasmosis (Hassanain *et al*., 2013).

Also 116 kDa was detected in RH *T. gondii* antigen using Toxoplasma infected chicken serum (Hassan *et al*., 2015). So, 116 kDa is behind the success of chicken *T. gondii* fraction in the diagnosis of human toxoplasmosis and participate in the diagnosis of chicken toxoplasmosis together with 83 kDa. The role of *T. gondii* infected chicken in the epidemiology of human toxoplasmosis is previously documented (Aboelhadid *et al*., 2013). Where, higher seroprevalence of human toxoplasmosis in poultry contacts (37.5%) was recorded than non-poultry contacts (30.5%). This is in addition to the fact that routes to human toxoplasmosis include ingestion of uncooked meat of animals or birds containing tissue cysts (Dubey *et al*., 2004, 2010). The capability of chicken *T. gondii* tachyzoites to infect human is partially explained, presently by the existence of common components between both isolates as 116 kDa. In the present study the isolated fraction was used in detection of chicken toxoplasmosis and proved 90.1%. Previously, a total rate of 68.8% was recorded in six Egyptian governorates by ELISA but using crude chicken tachyzoites antigen (Barakat *et al*., 2012).

Recently, this study recorded chicken toxoplasmosis percentage of 78.12% by ELISA, in which crude Toxoplasma tachyzoites antigen was adopted (Hassan *et al*., 2015). Differences in prevalence rates are attributed to multiple reasons as sample size, area of study, adopted antigens and assays in the diagnosis. Where, the isolated immunogenic fraction is more potent in diagnosis than crude extract and ELISA is more sensitive than MAT. The high infection percentage in the present study was matched with other results of Dubey (2010a) and Dubey *et al*. (2010), where they found that toxoplasmosis may be reached to 100% in backyard chicken. Also, in the present study affinity purified fraction succeeded in detecting Toxoplasma antibodies in 30 infected human serum samples with Toxoplasma, recording 100% sensitivity. While its specificity was 84.4%, where it crosses-reacted with Fasciola antibodies. The present results were in harmony with that previous results surveyed in Egypt by Toaleb *et al*. (2014) and Shaapan *et al*. (2015) recorded cross reactivity between *F. gigantica* and *T. gondii.* Moreover, this cross-reactivity may be due to presence of a band of molecular weight 86 kDa in *F. gigantica* somatic antigen (Maleewong *et al*., 1997) and a band of 115 kDa in *Fasciola hepatica* somatic antigen (Elsagheir *et al*., 2015).

CONCLUSION

The present study clearly authenticated partially purified chicken tachyzoites antigen proved high diagnostic activity of chicken and human toxoplasmosis with 100% sensitivity and 84.4% specificity.

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