

Evaluation of Purified Horse Derived Antigen in the Diagnosis of Human Toxoplasmosis

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Abstract: *Toxoplasma gondii* infection in humans is routinely assessed by serological means. Here, the authors attempted to isolate fraction (LAb) from *Toxoplasma gondii* crude antigen (LA) prepared from *Toxoplasma gondii* strain isolated from infected horse tissues. The diagnostic activity of the isolated fraction was compared with RH strain and commercial kit antigens for detection of human toxoplasmosis. A total of 90 human serum samples was investigated by ELISA using the three compared antigens. The results showed that isolated horse fraction displayed highest diagnostic efficiency (80%) compared with RH strain (63%) and commercial kit (70%) antigens. By SDS-PAGE, LA was resolved into 11 bands of molecular weights ranged from 23-207KDa. Five of them were detected in LAb with molecular weights ranged from (57-93KDa). Also, RH strain showed 11 bands of molecular weights (27-207 KDa). Immune-reactive bands of LA and LAb were identified by immunoblot assay using infected human sera. Two bands of 23 and 65 KDa were detected in LA. But LAb showed only one band of 65 KDa which may be responsible for its highest diagnostic potency. Collectively, purified local isolated fraction of *Toxoplasma gondii* from horse origin is one of the most promising antigens for adoption in routine screening of human toxoplasmosis.

Key words: *Toxoplasma gondii* • Horse • Antigens • ELISA • SDS-PAGE • Immunoblot

INTRODUCTION

Toxoplasmosis is caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*, which is able to infect most mammals and birds [1]. It is considered the most common food-borne parasitic infection requiring hospitalization [2]. It has been estimated that one third of the world population has been infected [3]. People are infected by inadvertent ingestion of oocysts or sporozoites in cat feces, or tissue cysts in undercooked infected meat or contaminated foods [4].

Several researchers have reported high prevalence of toxoplasmosis in domestic [5] and sports horses [6]. Consequently, infected horse tissues are considered as source of infection for people in country eating horse meat and an easy source for antigen preparation.

Laboratory diagnosis of *Toxoplasma* infection is usually based on the detection of specific antibodies. Many serological tests used in the detection of *T. gondii*-specific immunoglobulins are commercially available, the majority of which use native parasite antigens prepared from tachyzoites developed in mice and/or *in vitro* tissue culture [7-9].

Owing to the different sources or different collection and purification methods, the quantity and quality of antigens are often varied. Thus, the diagnostic system developed with such antigens is different in efficiency [10, 11]. In addition, production of commercial kits for diagnosis of human toxoplasmosis is laborious and expensive. Therefore, it is obviously necessary to produce large quantity of non expensive diagnostic antigen (s) to be utilized in large scale for the

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diagnosis of toxoplasmosis. So affinity purified antigen is considered the best alternative method to avoid these problems.

In previous studies in our laboratory, purified local strain antigen of *T. gondii* tachyzoites isolated from infected horse tissues was successfully used in the diagnosis of horse toxoplasmosis [12]. Meanwhile, purified fraction isolated from sheep tachyzoites proved good potency (76.9%) in the diagnosis of horse toxoplasmosis [13]. Moreover, crude local antigen isolated from horse origin tachyzoites was used in the detection of human toxoplasmosis and showed comparable results compared with the commercial kit [14].

Consequently, it is possible to successfully utilize tachyzoite antigen regardless its origin in the diagnosis of toxoplasmosis. Furthermore, utilization of active components of diagnostic antigen increases the accuracy of diagnosis. So, the present study was designed to isolate affinity purified *Toxoplasma gondii* fraction from crude antigen of horse origin to be used in the diagnosis of human toxoplasmosis. In addition, evaluation of kinetics of IgG human antibodies to horse purified fraction in comparison with RH strain and commercial kit antigens in random human serum samples by ELISA was undertaken.

MATERIALS AND METHODS

Isolation and Maintenance of *T. gondii* Tachyzoite from

Horse Tissues: Viable infective *T. gondii* tachyzoites were recovered from the peritoneal exudates of mice 6-8 days post inoculation with digested horse meat samples as procedures described by Sharma and Dubey [15] and Dubey and Beattie [16]. Both virulent RH and horse locally isolated strains of *T. gondii* were maintained by serial passages in mice in the Zoonotic Diseases Department, National Research Center, according to the method of Johnson *et al.* [17].

Antigen Preparation: Both RH and local strain antigen (LA) of *T. gondii* tachyzoites was prepared for ELISA test as the method described by Waltman *et al.* [18]. Briefly, tachyzoites were repeatedly freeze and thawed 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 amount was determined by the method of Lowry *et al.* [19].

Serum Samples: A total of 90 human serum samples had been submitted to clinic of Department of Parasitology at

Faculty of Medicine, Ein-Shams University, Cairo Egypt. Patients were of different ages, sexes and showed different clinical signs in the form of fever, arthritis, reproductive manifestations, eye disorders and abortion. Samples were labeled in serial numbers and stored at -20°C until use.

Immunoaffinity Purification of LA: Affinity purification of LA was performed as described by Fagbemi *et al.* [20]. In brief, *T. gondii* positive horse sera were obtained from the slaughtered horses (from which *T. gondii* tachyzoites were isolated). The positive sera were dialyzed against 0.1 M NaHCO₃ containing 0.5 M NaCl and 0.02% NaN₃ and coupled to Cyanogen-bromide Sepharose 4B (CNBr-Sepharose 4B) swollen beads by strictly following the manufacturer instructions. Bound fraction was eluted with 50 mM glycine and 500 mM NaCl pH 2.3 and designated LAb.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Proteins of LA, LAb and RH strain antigens were separately electrophoresed on SDS-PAGE according to the method of Laemmli [21]. After separation, the gel was fixed in 50% methanol and stained with silver stain according to the method of Wray *et al.* [22]. Molecular weight standards were electrophoresed on the same gel to calculate the relative molecular weights of the examined antigens.

Immunoblot Assays: Immunoblot assay was utilized to identify immunoreactive components recognized in horse crude and isolated fraction of *T. gondii* using infected human sera. The assay was carried out as the method described by Towbin *et al.* [23].

ELISA Kit: One package of a commercially available ELISA Kit (Clinotec® diagnostic toxoplasma IgG ELISA) was brought and stored at 2-8°C until used for detection of anti *T. gondii* IgG antibodies according to the instructions of the manufacture.

Enzyme Linked Immunosorbent Assay: Horse tachyzoite isolated fraction (LAb) and RH strain antigen were used in ELISA to detect IgG *T. gondii* antibodies in the collected human sera. The optimum antigen, serum and conjugate concentrations were determined by checkerboard titration and test procedures were carried out according to the method described by Lind *et al.* [24]. The cut off values of optical densities were calculated according to Hillyer *et al.* [25].

RESULTS

Immunoaffinity Purification: The purification process of LA yielded two distinct fractions; LAb and LAunb by CNBr-Sepharose 4B affinity column chromatography.

Electrophoretic Profile of LA, LAb and RH Strain of *T. gondii* Tachyzoites: The electrophoretic profile of the three types of *T. gondii* tachyzoite antigens showed that LA yielded 11 bands of molecular weights ranged from (23-207KDa). LAb revealed 5 bands of molecular weights ranged from (57-93KDa). RH strain was resolved into 11 bands with different molecular weights that ranged from 27-207 KDa (Fig. 1).

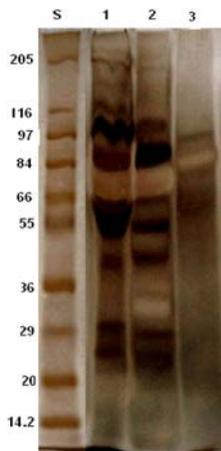


Fig. 1: Comparative electrophoretic profiles of *Toxoplasma gondii* RH strain Ag (Lane1), LA (Lane 2) and LAb (Lane 3). Molecular weight standards in KDa (Lane S)

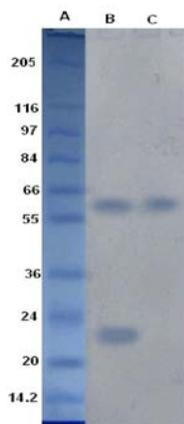


Fig. 2: Immunoreactive bands identified by positive human sera in horse local strain of *Toxoplasma gondii*. LA (laneB) and with isolated fraction from horse local strain LAb. (Lane c).Molecular weight standards in KDa (lane A)

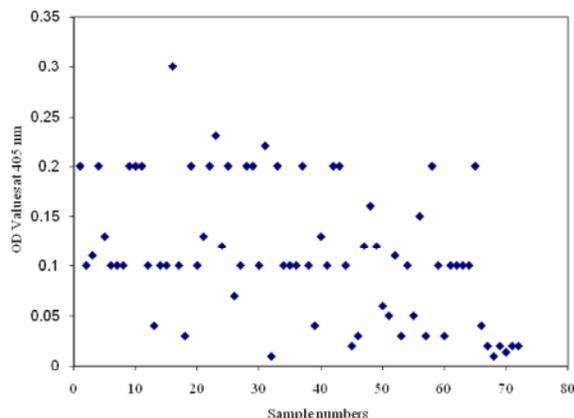


Fig. 3: Diagnostic potential of isolated fraction for human toxoplasmosis

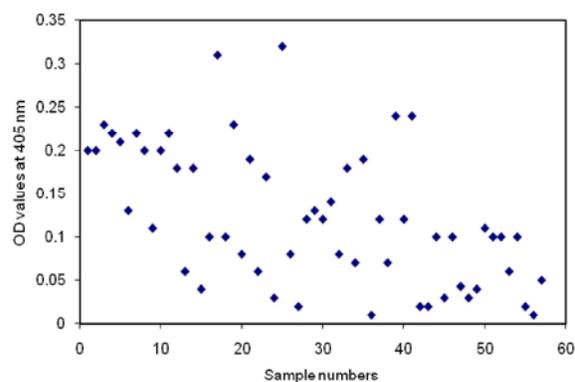


Fig. 4: Diagnostic potential of RH strain for human toxoplasmosis

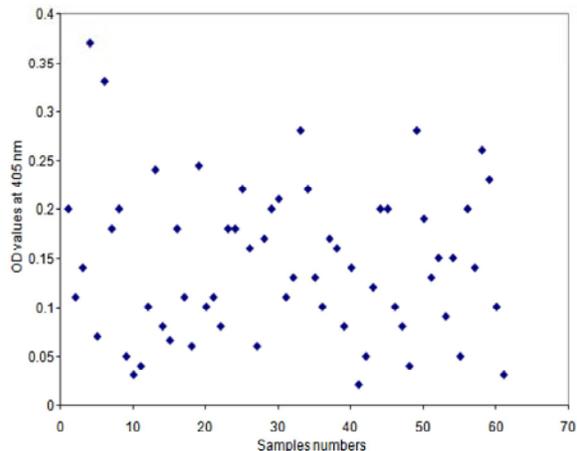


Fig. 5: Diagnostic potential of commercial kit for human toxoplasmosis

Immunogenic Bands: Identified immune-reactive bands with infected human sera in LA were 23 and 65 KDa, while one band of molecular weight 65 KDa was detected in LAb (Fig. 2).

Evaluation of Diagnostic Potencies of LAb, RH Strain and Commercial Diagnostic Kit Antigens by ELISA:

Figures (3-5) display that LAb was the most potent and showed the highest diagnostic potential (80%) followed by commercial kit (70%) and RH (63%) antigens.

DISCUSSION

Accuracy of serological diagnosis depends basically on the quality of utilized antigen. The quality includes in addition to diagnostic potentials, the availability and low costs of preparation. The current research adopts an approach of utilizing easily collected and comparatively low cost antigen in the diagnosis of human toxoplasmosis. Preparation of tachyzoite antigen from *Toxoplasma* infected horses is by far easier than from humans. But its diagnostic potential remains to be assessed in relation to currently used ones.

In a previous study in our laboratory, horse derived fraction was successfully utilized in the diagnosis of horse toxoplasmosis [12].

Currently, affinity purified antigen of horse origin was comparatively evaluated in the diagnosis of human toxoplasmosis. ELISA results showed highest diagnostic potency (80%) of the fraction compared with commercial kit (70%) and RH strain (63%) antigens. In another previous study in our laboratory, crude horse derived antigen was adopted in the diagnosis of human toxoplasmosis [14]. They proved that local strain antigen showed comparable results (49%) to commercial kit (45%). The present study could increase (80%) the previously recorded diagnostic potential of the crude extract (49%), by Shaapan and Hassanain [14], after isolation of immunogenic components using affinity purification process. Adoption of affinity column chromatography in optimizing the diagnostic potentials of antigens by isolating the most immunogenic components was previously reported either in toxoplasmosis (12, 13) or other diseases [26]. Isolation of immunogenic components can be adopted by other methods such as ion exchange chromatography [27]. They found that the electro eluted fractions by anionic exchange chromatography from crude tachyzoite antigen improve discrimination between acute and chronic human toxoplasmosis when used instead of the whole tachyzoites extracts.

In current research RH strain was resolved into 11 bands with different molecular weights ranged from (27-207KDa). Comparable number (13 bands) but with different molecular weight (23-154.5 KDa) were separated by Abdel-Rahman *et al.* [13]. Also, electrophoretic profile

of LAb revealed 5 bands of molecular weights ranged from 57-93KDa. Different profile of LAb was previously introduced by Ghazy *et al.* [12] with six bands of 27.5-177.9KDa. The difference in both profiles may be attributed to modifications in purification process or in preparation of antigen before loading to the gel.

Moreover, from the five bands of LAb, only one of 65KDa was identified by infected human serum samples in immunoblot which is mostly responsible for its high diagnostic activity. No literatures were available concerning the immunoreactive bands of horse tachyzoites isolated fraction with infected human sera. However, Ghazy *et al.* [12] detected four immunogenic bands of molecular weights (155.8-66.2 KDa) in horse tachyzoites LAb antigen with infected horse sera, which were responsible for diagnostic capabilities of horse toxoplasmosis. The difference in immunogenic bands of both fractions obtained may probably due to utilized antisera in each study either from human or horse.

The purified horse derived antigen of molecular weight 65KDa is one of the most promising antigens which could be used in routine screening of human toxoplasmosis and strongly addressed to be included in commercial diagnostic kits of toxoplasmosis in human.

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