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High Diagnostic Efficiency of Affinity Isolated Fraction in Camel and Cattle Toxoplasmosis

[1]Nagwa I. Toaleb, [2]Raafat M. Shaapan,
[1]Soad E. Hassan and [1,3]Faragalla M. El Moghazy

[1]Department of Parasitology and Animal Diseases,
Veterinary Research Division, National Research Center, Dokki, Giza, Egypt
[2]Department of Zoonosis, Veterinary Research Division,
National Research Center, Dokki, Giza, Egypt
[3]Department of Biology, College of Science and Humanity Studies,
Salman Bin Abdul Aziz Univ., K.S.A

Abstract: *Toxoplasma gondii* is a zoonotic protozoan coccidian parasite of both medical and veterinary importance worldwide. Sero-prevalence of *T. gondii* infection in camels and cattle using purified antigen isolated from the crude extract of camel *T. gondii* tachyzoites of local strain is the purpose of the current study. Affinity purification process resulted in isolation of a single specific fraction identified by hyper immune serum of rabbit coupled with CNBr-Sepharose 4B. By SDS-PAGE, the fraction was resolved into three bands of 84, 78 and 65 KDa compared with 12 bands associated with crude extract of 114, 92, 84, 78, 65, 52, 43, 33, 26, 22, 18 and 15 KDa. The isolated fraction showed more diagnostic potency compared with crude extract by enzyme linked immune sorbent assay (ELISA) using two fold serially diluted rabbit hyper immune serum. Serological screening of apparently healthy 60 camels and 94 cattle with the isolated fraction using ELISA revealed higher prevalence of toxoplasmosis in camels (66.7%) than in cattle (46.8%). In immunoblot assay two immuno-reactive components; 84 and 78 KDa of crude extract were recognized by camel naturally infected sera while, only one immunogenic band of 78 KDa was detected in isolated fraction. Naturally infected cattle sera identified two immunogenic bands of 65 and 22 KDa in crude extract. One of them, 65KDa, was identified in the fraction which is probably responsible for its high diagnostic potential. The results of the present work indicated that *T. gondii* infection is prevalent in cattle and camel, which may be a risk factor for human infection with *T. gondii*. Also, affinity isolated fraction of *T. gondii* from Local camel strain tachyzoite proved high diagnostic potency of cattle and camel toxoplasmosis.

Key words: *T. gondii* · Camel · Cattle · ELISA · Affinity Isolated Fraction

INTRODUCTION

Toxoplasmosis is one of the most important zoonotic diseases worldwide, caused by an obligatory intracellular parasite; *Toxoplasma gondii* which is a polyxenous pathogenic protozoan parasite belonging to phylum Apicomplexa. The parasite can infect humans and almost all warm-blooded animals [1]. *T. gondii* is responsible for major economic losses in most classes of livestock through abortions, still birth and neonatal losses. The distribution of this parasite depends on regions and weather condition of the environment where oocysts survive [2]. Besides vertical infection during pregnancy, humans can get infection post-nataly either by oral uptake of sporulated *Toxoplasma* oocysts or by ingestion of tissue cysts upon consumption of raw or undercooked meat of infected slaughtered animals which is considered as an important source of the infection for humans [3].

Corresponding Author: Raafat M. Shaapan, Department of Zoonosis,
National Research Center, Post Box 12622, El-Tahrir Street, Dokki, Giza, Egypt.
Tel: +20 2 25272439, Fax: +20 2 33371362.

Camel and cattle are from the most useful domestic food animals important to the economy of many countries particularly in the Africa and Middle East Regions. Beside their social and economic status, they play a very important role in the national income, as they are an important source of meat, milk and hide and constitute a major item in the livestock foreign trade list [4, 5].

Only a few *T. gondii* seroprevalence studies on camels exist. In Egypt, the infection rates among slaughtered camels were 46% using dye test DT [6], 17.4% using MAT [7] and recently 30.7% using MAT [8]. In Sudan, the prevalence of camel toxoplasmosis was 67% by latex agglutination test (LAT) [9]. In Saudi Arabia the infection rate was 16% by indirect haem-agglutination test (IHAT) [10] and in United Arab Emirates was 31.4% using ELISA [11].

The prevalence data of *T. gondii* infection in cattle at the different localities of the world are extremely variable. The prevalence was 5% in USA in beef cattle using MAT [12], 10.5% of dairy cattle in Vietnam using MAT [13], 20.8% in Switzerland using ELISA [14], 22.3% in Thailand by LAT [15], 71% in Brazil using IFAT [16], 32% in Sudan by LAT [17] and 5.7% in China by IHAT [3]. Little known surveys of *T. gondii* in cattle from Egypt revealed that the rate of infection was 16.7% using IHAT [18], 10.7% by indirect fluorescent antibody test IFAT [19] and recently, 22.7% by ELISA [20].

However, little is known about *T. gondii* types of isolated strains distribution in Egypt, especially in slaughtered animals which will be later processed for food. Therefore, the aim of this study was to purify isolated fraction antigen eluted from the crude extract of tachyzoites of local isolated *T. gondii* strain of camel origin through column chromatography and use this immunogenic potent purified fraction antigen for the investigation of camel and cattle toxoplasmosis by indirect ELISA.

MATERIALS AND METHODS

***Toxoplasma Gondii* Strain:** *T. gondii* local isolate was successfully obtained after many trails of bioassay of the suspected infected camel tissues in cat and mice as the procedures described by Shaapan and Ghazy [21]. The isolated camel *T. gondii* strain was maintained in the laboratory by serial passage in mice according to the procedures of Hafid *et al*. [22], briefly, about $2x10^6$ *T. gondii* tachyzoites were inoculated into 3 to 5 Swiss albino mice 1-2 month-old, the mouse peritoneal cavity washed with 5 ml sterile normal saline 2–3 days post inoculation. The peritoneal wash collected and examined microscopically for tachyzoites. Further inoculation was done every 2-4 days depending up on the ambient temperature and the condition of the inoculated mice.

Blood and Serum Samples: A total number of 60 and 94 blood samples were collected from apparently healthy camels and cattle intended for slaughtering at the main abattoir of Cairo (El-Bassatin), Egypt. Sera were individually separated, labeled and stored at -20°C until used for serological testing.

Preparation of Rabbit Hyper-Immune Serum: 40 ug of crude *T. gondii* tachyzoites antigen per kg of rabbit was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously into each of 5 rabbits [23]. A booster dose of the antigen in Freund's incomplete adjuvant was injected 14 days later. Second and third booster doses were given on days 21 and 28, respectively. Blood samples were collected 4 days post last injection from rabbit's ear vein and antisera were separated, aliquoted and stored at -20ºC until use.

Immune Affinity Chromatography: Affinity purification of local camel *T. gondii* strain was performed as described by Ahn *et al.* [24] with minor modifications. In brief, sera from hyper immune rabbit with *T. gondii* were dialyzed against 0.1 M $NaHCO_3$ containing 0.5 M NaCl and 0.02% NaN_3 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.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): Proteins of isolated fraction and crud local camel strain were separately electrophoresed on SDS-PAGE according to the method of Laemmli [25]. After separation, the gel was fixed in 50% methanol and stained with silver stain according to Wray *et al.* [26] and the relative molecular weights of the examined antigens were calculated.

Immunoblotting: Immunoblot assay was utilized as the method described by Towbin *et al*. [27] to identify the immune-reactive bands recognized in the both crude extract and isolated fraction of *T. gondii* tachyzoites of camel strain by naturally infected camel and cattle sera.

Enzyme Linked Immunosorbent Assay (ELISA): ELISA was firstly adopted to evaluate the diagnostic potency of both isolated fraction and crude local camel strain antigens against rabbit hyper immune sera then *T. gondii* antibodies in randomly collected cattle and camel serum samples were detected using the most potent evaluated antigen. The optimum antigen, serum and conjugate concentrations were determined by checkerboard titration and the test procedures was carried out as described by Lind *et al*. [28].

RESULTS

Affinity Purification and Electrophoretic Profile: The purification process resulted in isolation of a single specific fraction identified by hyper immune sera of rabbit coupled with CNBr-Sepharose 4B. The isolated fraction was characterized by SDS-PAGE which resolved into three bands of 84, 78 and 65 KDa compared with 12 bands associated with crude extract (114, 92, 84, 78, 65, 52, 43, 33, 26, 22, 18 and 15 KDa) (Fig. 1).

Diagnostic Potency of Antigens Using Rabbit Hyper-Immune Serum: The isolated fraction showed higher diagnostic potency than crude extract at all serum dilutions as proved by ELISA (Fig. 2).

Sero-Diagnosis of Camel and Cattle Toxoplasmosis Using the Isolated Fraction: Based on data of Fig 2 showing higher diagnostic potency of the isolated faction of *T. gondii* camel tachyzoites than crude extract, it was used for screening of camel and cattle toxoplasmosis by ELISA. The isolated fraction showed higher potency in diagnosis of toxoplasmosis in random collected camel sera 66.7% [40 out of 60] than in random collected cattle sera 46.8% (44 out of 94) (Table 1).

Immunogenic Bands of *T. gondii* Crude Extract and Eluted Fraction: Two immunogenic bands were identified in crude extract by naturally infected camel sera with molecular weights 84 and 78 KDa while, one band of molecular weight 78KDa was detected in the isolated fraction (Fig. 3). Using naturally infected cattle sera two immunogenic bands of 65 and 22 KDa were detected in crude antigen and one of these bands; 65 KDa was recognized in the isolated fraction (Fig. 4).

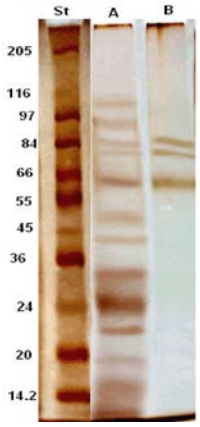

Fig. 1: Electrophoretic profile of crude extract (lane A), isolated fraction (lane B)and molecular weight standards (Lane St)

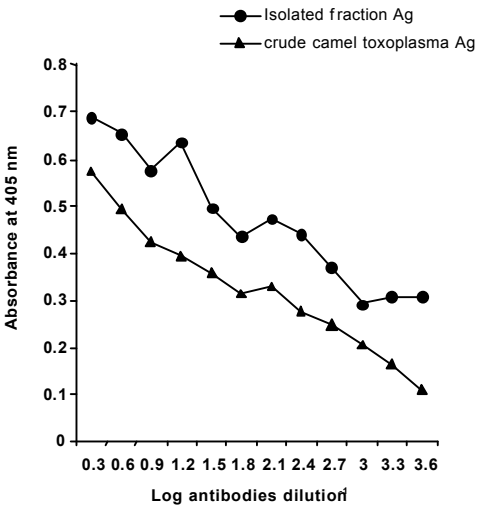

Fig. 2: Comparative diagnostic potentials of crude extract and isolated fraction of local camel strain antigen against rabbit hyper-immune sera.

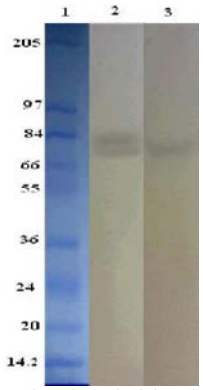

Fig. 3: Immune-reactive bands identified by immunoblot assay using naturally infected camel sera. Crude extract (lane 2), isolated fraction (lane 3) and molecular weight standards. (lane 1)

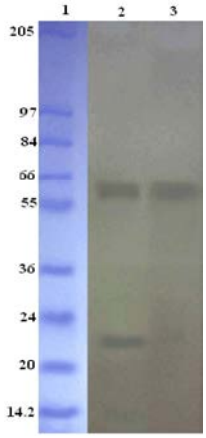

Fig. 4: Immune-reactive bands identified by immunoblot assay using naturally infected cattle sera. Crude extract (lane 2), isolated fraction (lane 3) and molecular weight standards. (lane 1)

Table 1: Sero-prevalence of camel and cattle toxoplasmosis by ELISA using *T. gondii* camel strain isolated fraction

		Positive sero-prevalence	
Sera	No. of samples examined	No	%
Camel	60	40	66.7%
Cattle	94	44	46.8%

DISCUSSION

Toxoplasmosis is routinely diagnosed by the detection of specific antibodies to *T. gondii* [29]. While in most previous serological tests crude antigen used in the detection of *T. gondii* antibodies, in the present study, after crude antigen of local camel strain tachyzoites was prepared the affinity purification process was performed to increase the diagnostic potency of crude extract by CNBr-Serpharose 4 B affinity column chromatography. Purification process resulted in one immunogenic fraction identified by rabbit's hyper immune serum. To our knowledge, the isolated fraction of local camel strain tachyzoites was utilized for the first time in Egypt in the detection of antibodies to *T. gondii* in farm animals (camels and cattle) using ELISA. The selection of ELISA in the present research was based on previous studies suggested ELISA as a good sensitive and specific tool for epidemiological surveys of *Toxoplasma* infection in animals and human [30, 31].

In current research, the isolated fraction proved success in the detection of *T. gondii* antibodies in 40 out of 60 investigated camels (66.7%). Our finding was higher than [11] in UAE who used ELISA plate coated with P30 (pure protein) but he used peroxidase –conjugated protein G as a secondary antibodies. In addition, Lower prevalence was detected by authors used crude extract and different techniques; 17.4% by direct agglutination tests [7] and 30.7% using MAT [8].

Isolation of *T. gondii* from tissues of sero-positive cattle has been generally unsuccessful [32]. In addition, isolated fraction of sheep *T. gondii* tachyzoites antigen was successfully utilized in diagnosis of horse toxoplasmosis [33]. Moreover, *T. gondii* horse isolated fraction proved good potency in diagnosis of human toxoplasmosis [34]. So, in the current study isolated fraction of the local camel strain tachyzoites was successfully utilized in the diagnosis of cattle toxoplasmosis. The fraction can detect 44 positive samples with *T. gondii* out of 94 investigated cattle (46.8%). In Egypt, lower prevalence (10.7%) was detected by Ibrahim *et al*. [19]. This difference in infection percentages probably attributed to utilization of surface antigen 2 of *T. gondii* (Tg SAG2t), while in the present study, affinity purified fraction was utilized. In addition, Hassanain *et al*. [20] showed that seroprevalence of *T. gondii* in cows was 22.7% using crude extract. Also, the current recorded prevalence was higher than that reported in many countries; 5% in USA [12], 10.5% in Vietnam [13], 20.8% in Switzerland [14], 22.3% in Thailand [15], 32% in Sudan [17] and 5.7%% in China [3]. This difference in the prevalence may be due to utilizing crude extracts in previous study, while, currently, isolated fraction was utilized. This agreed with Villavedra *et al*. [35] who concluded that the electro-eluted fraction of 30-33 KDa antigen improved diagnosis of human toxoplasmosis when used instead of the whole tachyzoites extract and also confirmed previous results, reported higher incidence of *T. gondii* in tested animals particularly with the use of affinity purified antigens [33, 34].

A comparable electrophoretic profile of isolated fraction with molecular weights 84, 78 and 65 KDa was obtained by SDS-PAGE. Only one immunogenic band of 78 KDa was detected in immunoblot assay using naturally infected camel sera, while one band of molecular weight 65 KDa was detected by naturally infected cattle sera. To our knowledge, no available literatures concerning immunoreactive bands of camel tachyzoites isolated fraction that were identified with camel and cattle naturally infected sera.

CONCLUSION

The high prevalence of toxoplasmosis in cattle and camels not only affects the development of the livestock industry but is also an important infective source for human toxoplasmosis. This study provides additional information of the prevalence of camels and cattle toxoplasmosis in Egypt and will assist in developing strategies for controlling this economic disease. Based on the available literatures, this is the first report concerned with the detection of *T. gondii* antibodies in camel and cattle by purified isolated fraction of *T. gondii* from Local camel strain and finally, the current research recommends utilization of this potent immunogenic isolated fraction in the detection of *T. gondii* antibodies in camels and cattle.

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