Global Veterinaria 12 (1): 140-145, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.01.8240

Adoption of Immuno-Affinity Isolated *Fasciola gigantica* Fraction for Diagnosis of Ovine Toxoplasmosis

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Abstract: A 65KDa cross–reactive fraction was isolated from *Fasciola gigantica* adult worm antigen (CrFgf) using CNBr Sepharose 4B affinity column chromatography coupled with anti-*Toxoplasma gondii* antibodies. Specific *T. gondii* fraction (STgf) was also collected after applying *Toxoplasma* crude extract to the column and revealed two bands of 90KDa and 65KDa using sodium dodecyle sulphate polyacylamide gel electrophoresis (SDS-PAGE). Importantly, CrFgf reacted positively with *T. gondii* rabbit hyperimmune serum using enzyme-linked immunosorbent assay (ELISA). It also showed comparable diagnostic potency of experimental sheep toxoplasmosis with STgf at both acute and chronic phases of infection. Moreover, the isolated fraction proved diagnostic potentials of natural toxoplasmosis after examining serum samples with commercialized latex agglutination *Toxoplasma* IgG (LAT), one step diagnostic rapid test. Of 96 analyzed sheep serum samples, 69 (71.88%) and 28 (29.17%) were *Toxoplasma gondii* positive by CrFgf ELISA and commercial test respectively. Out of 28 positive samples by commercial test, 26 were positive by ELISA recording 92.87% sensitivity of CrFgf ELISA in the diagnosis of ovine toxoplasmosis. The current results suggest CrFgf as a reliable antigen for the detection of *T. gondii* infection.

Key words: *F. gigantica* · Cross–Reactive Fraction · Ovine Toxoplasmosis · ELISA · LAT

INTRODUCTION

Sheep are important in many countries economics, as their products are a source of food or other benefits for humans. Sheep are commonly infected with *Toxoplasma gondii*. Clinical symptoms in sheep include early embryonic death and mummification, stillbirths, neonatal death or birth of alive but weak lambs [1]. The meat of infected sheep is a source of *T. gondii* infection for humans and carnivore animals [2, 3].

Infection in sheep can occur after consumption of contaminated feedstuffs or grazing land with sporulated oocysts [4] and transplacentally [5, 6]. Most infections in sheep occur following birth [7]. Some recent data suggest that in some circumstances persistently infected sheep may transmit the parasite to the fetus in subsequent pregnancies and abortions may occur [4, 8].

Transmission of *T. gondii* tachyzoites in unpasteurized sheep or goat milk and also may occur via tachyzoites contained in blood products, tissue transplants, but are not probably important epidemiologically in animals [9]. Indeed, the infection not only results in significant reproductive (and hence economic) loss but also has implications for public health and zoonotic transmission because of the consumption of infected meat or milk [3, 10].

Laboratory diagnosis of *T. gondii* infection is based on the detection of specific immunoglobulins by serological tests [11, 12]. ELISA seems to be the most practical and economic for detection of *T. gondii* antibodies and adapted for use in most domestic animals including sheep and goats [3, 12]. Although the detection of specific antibodies by ELISA using crude *T. gondii* antigens proved success, isolated fractions would be

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more accurate [13]. However, it is necessary to develop isolated reliable and sensitive parasite antigens for serological diagnosis.

A 65-kDa protein, originally identified on cysts and later found to be expressed on both bradyzoites and tachyzoites [14-16]. and also was documented to be immunogenic during infection with *T. gondii* in a mouse model and found to be a promising tool for the serodiagnosis of human toxoplasmosis [16-19]. The recombinant form of 65KDa was adopted by Tumurjav *et al.* [20] for diagnosis of ovine toxoplasmosis. Moreover, a 65KDa was included in affinity isolated fraction of the local camel strain tachyzoites and successfully utilized in the diagnosis of cattle toxoplasmosis in our laboratory [21] and on the other hand, Shaapan *et al.* [22] indicated that 65KDa *Fasciola gigantica* band was found to be cross-reactive with *T. gondii*.

In the present study, a 65KDa *F. gigantica* cross-reactive band will be isolated and its diagnostic potentials of experimental and natural ovine toxoplasmosis will be assessed.

MATERIALS AND METHODS

Antigen Preparation

Somatic Worm Antigen of *F. gigantica*: Adult flukes were homogenized in 0.1M Phosphate Buffer Saline (PBS) pH 7.2 supplemented with 8 mM phenylmethylsulfonyl fluoride (PMSF), as described by Oldham [23]. The homogenate was then subjected to ultra-sonication at maximum amplitude (peak to peak) for 10 min, two times with an interval of 1 min using an ultra sonicator (Misonix, USA) and centrifuged at 18,000 rpm for 45 min at 4°C. The supernatant was collected and designated as FgA.

***T. gondii* Tachyzoites Antigen:** Local *T. gondii* strain previously isolated from sheep tissues was maintained in Department of Zoonosis, National Research Center by serial passage in mice according to the method of Johnson *et al.* [24]. *T. gondii* tachyzoites antigen was prepared as described by Waltman *et al.* [25], briefly, tachyzoites were repeatedly freeze and thawed to rupture the parasite wall, sonicated and centrifuged at 18,000 rpm for 45 min at 4°C. The supernatant was collected and designated as TgA.

Determination of Antigens Protein Content: The prepared antigens were separately assayed for its protein content by the method of Lowry *et al*. [26], then aliquot and kept at -20°C until use.

Preparation of *T. gondii* Hyper Immune Rabbit Serum: 40 µg / kg of crude *T. gondii* tachyzoites antigen was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously into each of 5 rabbits [27]. 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 [28]. Blood samples were collected 4 days post last injection from rabbit's ear vein. Collected *T. gondii* hyper immune rabbit serum (TgHRS) was aliquoted and stored at -20°C until used.

Experimental Infection of Sheep with *T. gondii*: Two sheep of 18 month-old, were experimentally infected with local strain of *T. gondii* tachyzoites as the procedures described by Dubey and Beattie [29]. All animals were proved sera-negative for *T. gondii*-specific IgG using modified agglutination test (MAT) before experimental infection. Serum samples were collected in 0 day, 5 days, 2 months, 3months and 4months post infection. Serum samples were kept at –20°C for serological analysis and designated as TgISS.

Sheep Blood Samples: Total numbers of 96 blood samples were collected from sheep intended for slaughtering at the main abattoir of Cairo (El-Basateen). Serum samples were separated and stored at -20°C until use for serological testing.

Immunoaffinity Chromatography: Affinity purification of TgA and FgA was performed as described by Ahn *et al.* [30] with minor modifications. In brief, TgISS was dialyzed against 100mM $NaHCO_3$ buffer pH8.3 containing 500 mM NaCl and 0.02% NaN_3 and then coupled to Cyanogen Bromide-activated sepharose-4B (Sigma Chemical Co.) by strictly following the manufacture instructions. TgA and FgA were separately applied to the column and bound fractions were eluted using 50mM glycine containing 500mM NaCl.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): Proteins of four antigens; TgA, FgA and isolated fractions were separately electrophoresed on 10% SDS-PAGE, according to the method of Laemmli [31]. After separation, the gel was fixed in 50% methanol and stained with Silver stain according to the method of Wray *et al.* [32]. Molecular weight standards were electrophoresed on the same gel to calculate the relative molecular weights of the examined antigens.

Enzyme Linked Immunosorbent Assay (ELISA): The immunogeic value of two affinity purified fractions were assessed by ELISA using two fold serially diluted TgHRS. The diagnostic potentials of both fractions were monitored by ELISA using TgISS. *F. gigantica* fraction was adopted to detect natural ovine toxoplasmosis using ELISA. ELISA was performed according to Lind *et al.* [33] with little modifications. Antigens concentration, serum and conjugates dilutions were determined by checkerboard titration. The cutoff value was calculated by the method of Hillyer *et al.* [34].

Latex Agglutination *Toxoplasma* IgG Rapid Test (LAT): Nova Test, one step Diagnostic Latex Agglutination Colloidal Gold Chromatography Rapid Test (Cassette), based on the principle Gold Immuno-chromatography Assay (GICA) manufactured by ATLAS LINK (BETJING) was used as a confirmatory test to detect *T. gondii* IgG in 96 sheep serum samples. Recombinant *Toxoplasma* antigen was used to detect *Toxoplasma* IgG in serum specimens. The sample is considered positive when distinct color bands appear on both the control region and the test region. While in negative sample, only one band appears

Statistical Analysis: Data are expressed as mean ±SD. Comparison between the mean values of different optical densities was performed using 1way ANOVA test to compare diagnostic potentials of isolated fractions in *T. gondii* infection. The difference was considered statistically significant at $P \leq 0.05$.

RESULTS

Isolated Fractions: The Purification process produced two fractions; a single specific fraction of *T. gondii* (STgf) and cross-reactive fraction of *F. gigantica* (CrFgf).

Electrophoretic Profiles of Isolated Fractions: The electrophoretic profile of CrFgf revealed only one band of 65KDa, while STgf resolved two components of molecular weight 90KDa and 65KDa; whereas, the electrophoretic profile of FgA showed 14 bands ranged from 207 to 14 KDa and TgA resolved 8 bands ranged from 165 to 29 KDa (Fig. 1, Lane 2, 3, 4 and 5).

Diagnostic Potency of Isolated Fractions: Both CrFgf and STgf showed high diagnostic potency of experimental ovine toxoplasmosis. CrFgf showed significant higher potency in detecting *Fasciola* cross-reactive antibodies

Table 1: Serodiagnosis of ovine toxoplasmosis by ELISA with CrFgf and commercial LAT test

	ELISA		
Commercial LAT test	+ve	-ve	Total
+ve	26 (27.08%)	2 (2.08%)	28 (29.17%)
-ve	43 (44.79%)	25 (26.04%)	68 (70.83%)
Total	69 (71.87%)	27 (28.12%)	96 (100%)

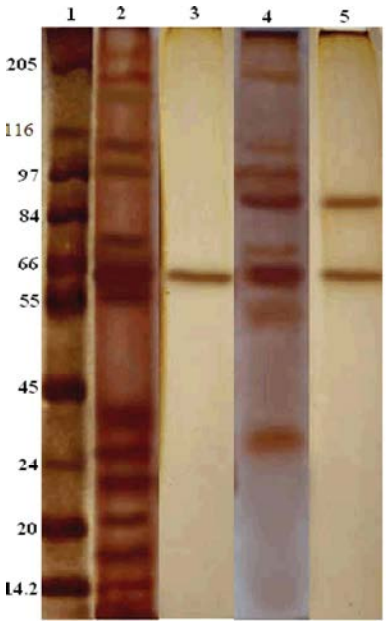

Fig. 1: Comparative SDS-PAGE profile of four antigens. Lane 2; FgA, Lane 3; CrFgf, Lane 4; TgA, Lane 5; STgf and Lane 1; Molecular weight standards in KDa

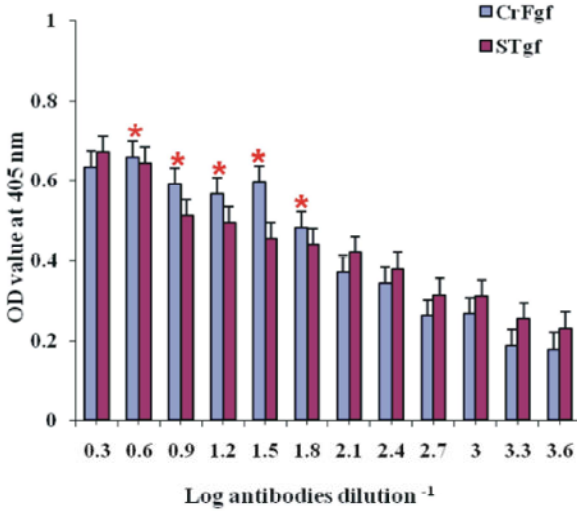

Fig. 2: Profile of antibody response of TgHRS towards CrFgf and STgLf. *The difference is significant when $P \leq 0.05$

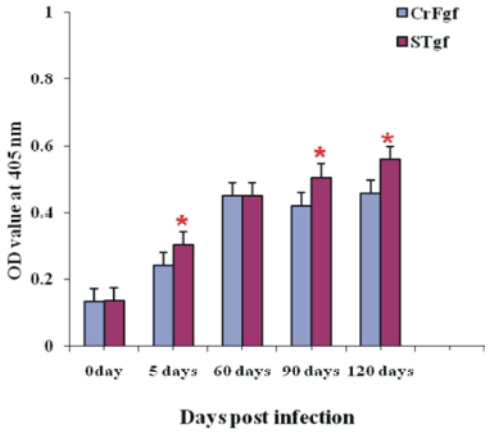

Fig. 3: Potency of CrFgf and STgf in IgG detection in experimentally infected sheep sera with *T. gondii*. *The difference is significant when $p \leq 0.05$

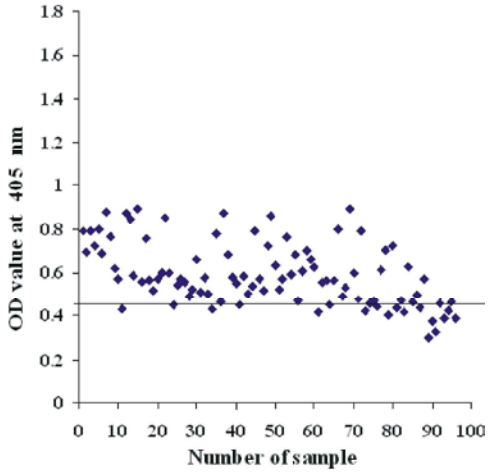

Fig. 4: Diagnostic potential of CrFgf for sheep toxoplasmosis by ELISA

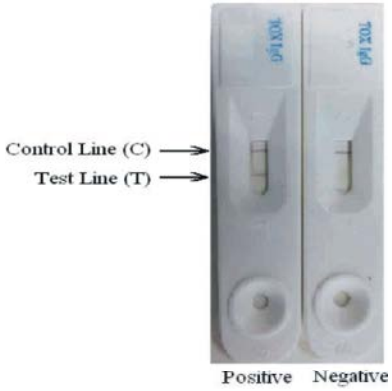

Fig. 5: Diagnosis of sheep toxoplasmosis by commercial diagnostic rapid test

with *Toxoplasma* than STgf at low dilution of TgHRS by ELISA (Fig. 2). The fraction also showed comparable diagnostic potency of experimental sheep toxoplasmosis to STgf until 2 months post infection (Fig. 3).

Diagnostic Potency of CrFgf ELISA in Ovine Toxoplasmosis Compared with Commercial LAT: The diagnostic potency of CrFgf for sheep toxoplasmosis detected by ELISA and also using commercial LAT assays (Fig. 4 and 5). The results of analyzed 96 serum samples, 69 (71.87%) and 28 (29.17%) were positive by CrFgf ELISA and commercial LAT test, respectively. Of 28 positive samples with commercial LAT test, 26 (92.86%) were positive by CrFgf ELISA (Table 1).

DISCUSSION

In the present study, we have evaluated the usefulness of the 65-kDa CrFgf for the diagnosis of ovine toxoplasmosis. The CrFgf reacted with hyper immune rabbit sera raised against *T. gondii*, indicating that CrFgf has good antigenicity for detecting antibodies to *T. gondii*. Moreover, sheep experimentally infected with *T. gondii* developed detectable antibody responses to CrFgf as early as 5days and 2 months post-infection and maintained these responses until 4 months post-infection. These results proved that ELISA with CrFgf could detect antibodies to *T. gondii* in both acute and chronic stages.

The high potency of CrFgf in the detection of infection in sheep experimentally infected, combined with its reported diagnostic potential in hyper immune *T. gondii* rabbit sera, is motivated us to use ELISA with CrFgf for the detection of natural ovine toxoplasmosis. Collected sheep serum samples were examined for the presence of antibody to *T. gondii* by ELISA with CrFgf and the results were compared with those obtained from the commercialized *Toxoplasma* IgG Rapid Test. 69 (71.87%) and 28 (29.17%) samples were positive by the ELISA and commercial test, respectively. These results revealed the high sensitivity of CrFgf ELISA in the detection of ovine toxoplasmosis (92.86%).

The higher positive proportion was detected by the ELISA than by the commercial test can be explained by the constant expression of a 65 KDa in tachyzoites and bradyzoites. 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 [35]. Therefore, the aim of the current study was to use easy prepared, cheap and potent antigen for detection of *T. gondii* infection in sheep using indirect ELISA.

In the present study, selection of the antigen depended on cross-reaction as a common phenomenon among parasites [36, 37]. It also depended on a previous study in our laboratory proving co-existence of 65KDa in both parasites [22]. Putting in consideration that collection of *Fasciola* worms from infected buffalo livers is easier and cheaper than *T. gondii* tachyzoites. The concept of adoption of one parasite antigen in the diagnosis and protection against other infection was previously reported [38-40]. The most common model for this approach was repeatedly reported between *F. hepatica* and *Schistosoms mansoni* [41]. Another approach for adoption of 65KDa was the use of its recombinant form that successfully utilized in the diagnosis of ovine toxoplasmosis by indirect ELISA [20].

In conclusion, the results of the current study demonstrated the usefulness of CrFgf; the cross-reactive antigen between *F. gigantica* and *T. gondii* as a diagnostic antigen for ovine toxoplasmosis. Further study is needed to evaluate the usefulness of ELISA with CrFgf for the detection of *T. gondii* IgM.

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