Journal of the Egyptian Society of Parasitology, Vol.46, No.2, August 2016
J. Egypt. Soc. Parasitol. (JESP), 46(2), 2016: 441 - 452

# DOT-ELISA AS A FIELD TEST FOR HYDATID DIAGNOSIS

By
HANY M. ABOU-ELHAKAM[1*], ALYAA A. FARID[1], NOHA A. MAHANA[1], IBRAHIUM R. BAUIOMY[2] AND AZZA M. ELAMEER[1]
Department of Zoology[1], Faculty of Science, Cairo University and Department of Parasitology[2], Theodor Bilharz Research Institute (TBRI), P.O. Box 30 Imbaba, Giza, Egypt (*Correspondence: hlongous@yahoo.com)

## Abstract

Cystic hydatid disease (Hydatidosis) is one of the most important parasitic zoonoses and remains a public health and economic problem all over the world. Cyst fluid was obtained from hepatic and pulmonary cysts for demonstration of protoscolices and hooklets. Therefore, a standardized and approachable diagnostic tool for the serodiagnosis of CE is still needed. Dot-ELISA is a solid phase diagnostic method for detection of antigen or antibody that is used widely for diagnosis of protozoan and metazoan diseases of human and animals. In the present study, *E. granulosus* protoscolex antigen was early detected in patient sera using Dot-ELISA, PAb was prepared from anti-rabbit sera and used for coating and as conjugate in Dot-ELISA technique. 48 patients out of 50 were positive to *E. spp.* with sensitivity and specificity 96% and 94%, respectively. The PPV was 94% and NPV was 90%. Finally, the present results showed that the Dot-ELISA was easy to perform, not expensive, safe, and with good sensitivity and specificity.

**Key words:** Egypt, Cystic echinococcosis, Diagnosis, Dot-ELISA, pAb

## Introduction

Human Cystic Echinococcosis (CE) is a chronic endemic helminthic disease which is caused by the metacestode (larval) stage of the dog tapeworm *E. granulosus* (Zhang *et al*, 2006; Eckert *et al*, 2011). It is an important public health problem worldwide and one of the most important zoonotic diseases in Egypt. Herbivores such as sheep, horse, cattle, pig, goat and camel are the intermediate hosts. The highest percentages of fertile cysts were detected in sheep and goat, so both being the most important intermediate hosts for *E. granulosus* (Rady *et al*, 2014)

The liver and the lungs are the most commonly involved organs but the cyst can occur almost anywhere in the body, in the *E. granulosus* vital cycle the adult tapeworm lives in the intestine of some carnivores (definitive hosts), and larval stage develops in the herbivores (intermediate hosts) and humans, essentially in liver and lungs (Oudni-M'rad, 2015).

The definitive hosts of *E. granulosus* are carnivores such as dogs and wolves, which are infected by ingestion of offal containing hydatid cysts with viable protoscoleces. After ingestion, the protoscoleces evaginate, attach to the canine intestinal mucosa, and develop into adult stages. Sexual maturity (length of 3-6 mm) is reached 4-5 weeks later (Izol *et al*, 2012).

*E. granulosus* protoscolex soluble somatic antigens (PSSAs) were assessed for their prognostic value in the serological follow-up of young patients treated for CE, compared to conventional hydatid fluid (HF) antigen (Nouir *et al*, 2008).

The germinal layer cells are responsible for secreting the laminated layer, the hydatid fluid that fills the cyst and also differentiate to the infective stage, a structure called protoscolex (PSC). When PSC is ingested by the definitive host, it evaginates and attaches to the small intestine, completing the cycle (Siracusano *et al*, 2012). The PSC is an ideal sample to analyze because it has three characteristics: it is not directly infective to humans; it can be studied with minimal host protein contamination and it can generate both the adult worm and new hydatid cysts under appropriate conditions (Riesle *et al*, 2014; Hidalgo *et al*, 2016).

Serological tests offered advantages over radiological procedures, including early diagnosis of infection (Zhang *et al*, 2012; Pagnozzi *et al*, 2014), where serological

tests for diagnosing hydatid infections in people living in endemic areas are useful because of the ease of performance and low cost. On the other hand, radiological techniques are often expensive or are not available in many endemic areas. The presence of raised specific antibody titers in patients with CE was assayed by various techniques such as indirect hemagglutination or latex agglutination, immunoelectrophoresis, complement fixation, immunoenzymatic and indirect fluorescent antibody tests (Soliman *et al*, 2014).

Ordinary serological tests such as immunoelectrophoresis, double diffusion in agar, or indirect hemagglutination were replaced by more sensitive assay methods such as enzyme linked immunosorbent assay (ELISA), immunoblot (IB), and indirect immunofluorescent antibody test (IFA). The ELISA is a high-sensitivity test and strongly recommended for the detection of specific antibodies in cystic human disease (CHD) cases (Koura *et al*, 2015).

The Dot-ELISA was the most sensitive serological tool which might be of value in serodiagnosis of human hydatidosis, where, Dot-ELISA technique has advantages which are of low cost, quick and does not require highly skilled personals. Moreover, the Dot-ELISA is a sensitive serological tool that might be valuable in serodiagnosis of human hydatid to complement and confirm the imaging diagnosis (Rady *et al*, 2014).

This study aimed to evaluate the most popular techniques, Dot-ELISA for diagnosis of human cystic echinococcosis *granulosus* (CE) by the circulating crude protoscolex antigen (CPA) detection.

## Materials and Methods

Animals: The New Zealand white rabbits, weighting approximately 3Kg. and about 4 months age, were used in the production of the antibodies.

Parasites: Hydatid cysts were removed from sheep and camel liver from Cairo Governorate Abattoir and were immediately transferred to the laboratory in TBRI in Hanks' buffer.

Preparation of parasite antigens: *E. granulosus* hydatid fluid was collected from ovine fertile cysts for subsequent use as a specific parasite antigen. Hydatid fluid was clarified by centrifugation at 10,000×g at 4°C for 60 min, dialyzed against phosphate buffer saline (PBS) pH 7.2, 10-fold concentrated with a collodion bag ultrafiltration apparatus (Sartorius GmbH, Gottingen, Germany) and lyophilized until use. Protoscoleces were prepared following the method of Rafiei and Craig (2002). In brief, protoscoleces were collected from fresh fertile sheep and camel liver cysts. Viability was determined by the vital coloration approach with 0.2% eosin staining. The protoscoleces were subjected to three cycles of freezing and thawing before being washed three times, each time by centrifugation (600×g for 5 min) and suspension in 10 times their volume of 0.15 M PBS, pH 7.2. Subsequently, the protoscoleces were suspended in four times their volume of the PBS containing 0.1mg aprotinin/ mL, then sonicated on ice in a 150 W ultrasonic disintegrator, in 10-s bursts with 5-s intervals, until no intact protoscoleces were microscopically visible. The sonicate was left standing on ice for one hr, then sedimented at 10,000×g for 30 min and the supernatant solution was split into aliquots and stored at -20°C until further processing.

Purification by DEAE-Sephadex G-5o and G-200 ion exchange chromatography: DEAE Chromatography is an effective method for separating proteins based on their charge. The DEAE group maintains a constant positive charge that is neutralized by counter ions, usually chloride ions. Other anions are capable of competing for the positive DEAE group (Sheehan and Gerald, 1996).

The specific fractions were analyzed for final max. purification by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), then protein content was colormetrically determined by the dye binding protein assay (Bradford, 1976) using com-

mercially available Bio-Rad kits (Bio-Rad laboratories, Richmond, CA, USA).

Production of polyclonal antibodies

Immunization of Rabbits for production of polyclonal Antibodies: Rabbits' anti-*Echinococcus* serum was obtained by immunizing New Zealand white rabbits intramuscularly with 1mg of protoscoleces antigen/each in equal volume of complete Freund's adjuvant (CFA). Two weeks later after the priming dose, each rabbit was a booster dose of 0.5 mg antigen emulsified in incomplete FA (IFA). The first boosting was two week after priming dose. The following 2 boosting doses (0.5 mg antigen) were given at weekly intervals in IFA. The rabbit was bled for collection of serum one week later after a preliminary testing of titer by indirect ELISA. Rabbit serum which contained the anti-*Echinococcus* pAb was fractionated and kept at -20°C.

Purification of rabbit anti-*Echinococcus* serum: Rabbit IgG purification steps were based on two different methods: Ammonium sulphate precipitation method (Nowotny, 1979) and Caprylic acid purification method (Mckinney and Parkinson, 1987).

Assessment of reactivity of target antigens of *E. granulosus* by indirect ELISA: This method was performed, with some modifications from that method of Engvall and Perlmann (1971).

Application of the polyclonal antibodies:

Study population: This study was conducted on 50 *E. granulosus* infected patients from highly endemic areas in Alexandria Governorate, out Patients Clinic and Hospital at TBRI and Al-Azhar University Hospital diagnosed by Endoscopic retrograde cholangiopancreatography (ERCP). Twenty five patients infected with other parasites (*S. mansoni*, Hook-worm and *F. gigantica*) were included as positive control. In addition, 20 individuals of the medical staff at TBRI served as parasite free-healthy negative control. In sterile polypropylene tubes, 5ml blood was taken by vein puncture, and was allowed to clot at room temperature for 2 hr. Serum was separated by centrifugation at 2000 g for 10 min., fractionated into small aliquots and stored at -70°C until use.

Conjugation of polyclonal Antibodies with Horse-Radish Peroxidase (Periodate Method): Periodate treatment of carbohydrates opens the ring structure and allows these moieties to bind free amino groups. Schiff's bases that have been formed are reduced by sodium borohydride (Nakane and Kawaoi, 1974; Tijssen and Kurstak, 1984).

Five mg of horse-radish peroxidase (HRP) enzyme was resuspended in 1.2 ml of dist. $H_2O$. 0.3ml of freshly prepared sodium periodate was added and incubated at room temperature, for 20 min. The HRP solution was dialysed versus 1 mM sodium acetate buffer, pH 4 at 4°C with several changes overnight. After dialysis the pH was increased to 9.5 by adding approximately 20 µL of 0.2 M $NaHCO_3$ buffer, pH 9.5. The HRP was removed from dialysis tubing and was added to 0.5 ml of the Ab (protein content 3mg/ml) solution. This was left to incubate at room temperature for 2hr. Hundred µl of 20 mM sodium borohydride (in 0.01 M PBS, pH 7.2) solution was added and the solution was incubated at 4°C, for 2hr. The HRP-mAb conjugate was dialysed versus 0.1 M borate buffer at pH 7.2 overnight at 4°C then collected in aliquots and saved at -20°C.

Dot-ELISA (Antigen Detection Assay):

Principle: Dot-ELISA was performed according to Boctor *et al.* (1987), using Biodot apparatus (BIO-RAD) for detection of protoscoleces antigens by double antibody sandwich procedures. The sandwich-dot ELISA test depends on the binding of anti-protoscoleces pAb to a sensitive surface of nitrocellulose membrane (NC) as a capture matrix.

The antigen under test binds the antibody (in serum); the amount bound was estimated by binding of a second antibody coupled to an active enzyme. Upon incubation with a suitable chromogenic enzyme substrate, the amount of second (conjugated) antibody binding was directly proportional to the

amount of substrate reaction, as measured by visual reading of the color intensity.

Optimization of the test: Bio-dot apparatus was used with dot format sample template connected to vacuum source. It has 96 wells, each with diameter 3 mm. arranged in 8 rows and 12 columns. NC was used as a capture matrix, 9×12 cm sheet with a pore size 0.2 micron (Bio-Rad).

The pre-wetted NC membrane was transferred to the Bio-dot apparatus and washed once with coating buffer for 5 min. After removing the excess solution, by suction, the membrane coated with 10-50 µl/well IgG pAb diluted in carbonate buffer (1/250, 500 and 1000), from original concentration (8 mg/ml), then incubated for variable times. Excess solution was removed, and then membrane was washed 3 times with 100 µl PBS-T/well. Then, blocking solution was applied (10-50 µl/well), incubated at room temperature for 15-45 min. The positive and negative control reference samples were added diluted 1/1-1/32 in the diluent-blocking buffer then incubated for variable times (15-45 min.) and washed 3 times with 100µl PBS-T/well. HRP conjugated pAb was used in 3 dilutions (1/100, 250 & 500) diluted in the diluent-blocking solution and incubated for variable times, then the NC membrane was removed from the Bio-dot apparatus and washed 5 times with 100 µl PBS-T/well each time, followed by 2 times washing with PBS only. DAB substrate was applied by immersing NC membrane in substrate solution. The reaction was stopped, just after the development of the color, with cold distilled water.

Key Features in Reliability of Test Results: Assay or test specificity and sensitivity can be selected and adjusted to meet the needs of a clinician for the diagnosis and monitoring of a disease. This may be accomplished by changing the selection of the reference value (i.e., cut-off or upper limit of normal) for the particular test (Zane, 2001).

## Results

Estimation of total protein content of protoscoleces antigen: The protoscoleces antigen obtained from hydatid cyst fluid contains 8mg/ml of total protein as measured by the Bio-Rad protein assay while it was 4.6 mg/ml after precipitation.

Gel electrophoresis for protoscoleces antigen. The SDS-PAGE analysis of protoscoleces antigen was resolved by SDS-PAGE (12.5%) under reducing condition and stained with Coomassie Blue. Protein bands were appeared at 27.5, 50 and 65 kDa which representing protoscoleces antigen (Fig. 1).

Characterization of protoscolex antigen: Reactivity of protoscoleces antigen of *E. granulosus* by indirect ELISA. The antigenicity of protoscoleces antigen was tested by indirect ELISA technique. Serum samples from patients infected with *E. granulosus* gave a strong reaction against protoscoleces antigen with mean OD reading equal to 1.21±0.13 (Tab.1). Production of anti- *E. granulosus* IgG pAb against protoscoleces antigen. Test blood samples were withdrawn from New Zealand white rabbit before the injection of each immunizing dose. They were tested for the presence of specific anti-*E. granulosus* antibodies by indirect ELISA. An increasing antibody level started 1 wk. after the first booster dose. Three days after the 2$^{nd}$ booster dose immune sera gave a high titer against protoscoleces antigen with OD of 2

Purification of rabbit anti-*E. granulosus* IgG pAb. The IgG fraction of rabbit anti-*E. granulosus* antibody was purified using different purification steps including ammonium sulfate precipitation method (Nowotny, 1979) followed by 7% caprylic acid precipitation method (Mckinney and Parkinson, 1987) and finally by DEAE-Sephadex A50-ion exchange chromatography. The total protein content of crude rabbit serum containing anti- *E. granulosus* antibody was 12.5 mg/ml. The yield of purified anti- *E. granulosus* IgG pAb following each purification step was determined by the assess-

ment of protein content. Using the 50% ammonium sulfate precipitation method, the protein content was 4.4 mg/ml, while following 7% caprylic acid precipitation method the content dropped to 2.6mg/ml and finally, the protein content of highly purified anti- *E. granulosus* IgG pAb subjected to ion exchange chromatography method (DEAE sephadex A-50 ion exchange chromatography) was 2mg/ml. The $OD_{280}$ profile of the antibody fractions obtained following purification by DEAE Sephadex A-50 ion exchange chromatography. The eluted antibody is represented by a single peak with maximum OD value equal to 1.245 at fraction number 7.

Characterization of the anti-*E. granulosus* pAb by SDS-PAGE. Analysis of 50% ammonium sulfate-precipitated proteins by 12.5% SDS-PAGE under reducing condition showed that precipitated proteins appeared as several bands. The purity of IgG after each step of purification was assayed by 12.5% SDS-PAGE under reducing condition. The purified pAb IgG was represented by H- and L-chain bands at 50 and 31 kDa, respectively. The pAb appears free from other proteins (Fig. 2).

The reactivity of anti-*E. granulosus* pAb against protoscoleces antigen and other parasitic antigens (*Fasciola gigantica*, *Toxoplasma gondii*, *Cryptosporidium parvvm)* was determined by indirect ELISA. The produced anti-*E. granulosus* pAb was diluted in PBS/T buffer gave a strong reactivity against protoscoleces antigen. The OD reading at 492 nm for *E. granulosus* was 1.94 compared to 0.23, 0.14 and 0.16 for *F. gigantica*, *Toxoplasma gondii* and *Cryptosporidium parvum*, respectively (Tab.2).

Dot-ELISA: The sensitivity of the Dot-ELISA techniques in thr detection of *E. granulosus* antigens in human was given (Tab. 3) and the sensitivity, specificity, PPV and NPV percentage of Dot-ELISA for detection of protoscoleces antigen in sera were also given (Tab. 4).

## Discussion

Human cystic hydatid disease (CHD) caused by the larval stage of the dog tapeworm, *E. granulosus*e, is a major infection with worldwide distribution and variable geographical incidence (Craig *et al.*, 2003). It is endemic in sheep raising areas and is among the most neglected diseases in the world today (Brunetti and Junghanss, 2009; Brunetti *et al,* 2010). Diagnosis is important not only for detection of cases but also for surveillance of the disease in the community and then for monitoring the impact of a control program for the disease in an area (Biffin *et al.* 1993). For the time being, specific diagnosis of CHD is based on immunological methods supplemented with radiological and ultrasound examinations.

Immunodiagnosis can also play an important complementary role in diagnosis of human echinococcosis (Rogan and Craig, 2002). It is useful not only in primary diagnosis but also in follow-up after surgical or pharmacological treatment. Additional advantages of immunodiagnosis include screening of large populations in communities from endemic areas, rapid testing of individuals in remote areas where imaging equipment may not be readily available, for follow-up monitoring of subjects in endemic areas, and for confirmation of CE or AE cases when physical imaging did not provide a definitive diagnosis (Qaq-ish *et al*, 2003; Hernandez *et al*, 2005).

Almost all traditional immunodiagnostic methods as complement fixation test, indirect haemagglutination test, indirect immunofluorescence antibody test, immunoelectrophoresis, and latex agglutination test), have now been replaced by the enzyme-linked immunosorbent assay (ELISA) and/or immunoblotting which are commonly performed in routine laboratory diagnosis of human echinococcosis (Rogan and Craig, 2002; Craig *et al*, 2003). The ELISA proved to be the test of choice for the diagnosis of CE in humans and particularly in the follow up of surgical cases using hydatid antigens

(Nasrieh and Abdel-Hafez, 2004). ELISA and immunoblotting are also considered as very useful laboratory tests for human echinococcosis. ELISA is a rapid and cheap immunological method that can be used for initial diagnosis of clinically suspected CE or AE, and that could also be applied in community screening, which could be extremely convenient. Rapid serological test formats such as dot-ELISA have been assessed for both human CE and AE (Feng *et al*, 2010).

The present study evaluated the most popular techniques, Dot-ELISA for the diagnosis of human Cystic echinococcosis (CE) by circulating crude protoscolex antigen (CPA) detection. Protoscoleces antigen was prepared from hydatid fluid that collected from ovine fertile cysts. For clinical practice, crude antigen has a high sensitivity, ranging typically from 92% to 98%.The protein content of the produced protoscolex antigen was 4.6 mg/ml. This procedure was followed by SDS-PAGE (12.5%) analysis. The use of this method proved previously to yield a highly antigen fraction as demonstrated (Sheehan and Gerald, 1996). Many bands were appeared in the SDS-PAGE analysis of protoscolex antigen, the most prominent thatwere 27.5, 50 and 65 kDa. Then the antigenicity of the crude antigen was tested by indirect ELISA.

Antigen detection was far more superior to antibody detection test as they provided a specific parasitic diagnosis for active infection (Chaya and Parija, 2013). Detection of parasite antigen also helps to demonstrate the effect of treatment, and has a high specificity (Van Dam *et al.*, 2004). The source of the antigens for the serological tests was fertile crude cyst fluids collected from naturally infected sheep (Golassa *et al*, 2011; Pagnozzi *et al*, 2014). The detection of Ag gives highly specific, yielding 90 and 95% specificity, respectively, for camel and sheep CE infections (Tabar *et al*, 2012). The detection of the circulating antigen may not only help in the diagnosis of an active infection, but also in the prognosis of the cases. Studies have indicated that the detection of the circulating antigen is useful in detecting hydatid disease in antibody-negative patients and also in assessing the status of the infection (Sunita *et al*, 2011). Serum antigen detection may also be less affected by hydatid cyst location and provides a tool for serological monitoring of antiparasitic therapy. Circulating antigen (CAg) in CE patient sera, can be detected using ELISA directly or indirectly (Zhang *et al*, 2012).

In the present study, protoscoleces antigen used for immunization of rabbit for preparation of rabbit IgG pAb. 1mg of protoscolex antigen was given to each rabbit in entire course of immunization in the first dose [1mg protoscolex antigen mixed 1:1 in complete Freund's adjuvant (CFA) (Sigma)] and 0.5 mg emu-lsified in IFA in the second and third booster doses injection. The first boosting was two wk after priming dose. The following boosting doses were given at weekly intervals according to Kamel *et al.* (2013). The purification procedures followed in this study were satisfactory, for IgG pAb three purification methods undertaken, ammonium sulfate precipitation which showed that, most of albumin was removed from rabbit anti-echinococcal IgG pAb, 7% caprylic acid. The purity of IgG pAb was assayed by 12% SDS-PAGE. The purified IgG pAb was represented by H- and L- chain bands at 53 & 31 kDa respectively, indicating that, the purified pAb appears free from other proteins. The present results agreed with El Amir *et al.* (2012) that SDS-PAGE analysis of the produced pAb, showed two different bands H- and L-chain at 53 & 31 kDa, respectively. The yield of pAb as protein content by these methods was 2.3 mg/ml IgG from starting protein content of 12.5 mg/ml (Yang and Harrison, 1996).

Simsek and Koroglu (2004) reported that SDS–PAGE analysis of hydatid cyst fluid showed that 6 specific protein bands at molecular weights 29, 45, 58, 68, 98 &116 kDa (Sambrook *et al,* 1989). These bands were

also reported by Kanwar and Vinayak (1992). The 68 &116 kDa bands had been detected by some researchers (Burgu *et al*, 2000).

The pAb has clear technical advan-tages, large quantities of pAb were produced from serum of an immunized animal. High affinity pAb can be isolated merely 2-3 months after initial immunization, so facilitated their rapid study. The pAb contains entire antigen-specific antibody population; offers a statistically relevant idea the overall picture of an immune response (Lipman *et al*, 2005).

Many immunologic tests based on antigen detection (Robijn *et al,* 2007; Sulbarán *et al,* 2010) and molecular or proteomic diagnostic techniques (Lier *et al,* 2009) were studied. But, an affordable, easy-to handle, sensitive and specific method was not yet available (Engels *et al,* 2002). Modified Dot-ELISA is antigen-antibody reaction done on nitrocellulose in polystyrene plate established to detect *T. gondii* antigens and antibodies. This test was sensitive and easy as compared standard ELISA and without special equipment (Liu *et al*, 2015).

Dot-ELISA was used by some authors to detect *Toxoplasma* antigen or antibody. Yamamoto *et al.* (1998) used it for detection of IgG, IgM & IgA antibodies against excreted-secreted (E/S) antigens of *T. gondii*. In their study E/S antigens from peritoneal exudates of infected mice were precipitated with 40% ammonium sulphate and then were used in immune-blot assay and Dot-ELISA. So the present research is planned to standardize Dot-ELISA, which is simple to perform and doesn't need expensive equipment to detect IgG and IgM specific antibodies against *T. gondii* compared with the IFA test and to evaluate the prevalence of toxoplasmosis in the elementary students.

Mehrabani *et al.* (2012) used Dot-ELISA to detect antibodies against *T. gondii* in human sera compared with IFA test. Out of 234 serum samples tested by IFA, 106 samples were positive for IgG, at titer of 1:64 & 53 samples were positive and 181 were negative by Dot-ELISA. Also, of 53 Dot-ELISA positive sera tested by IFA, 45 samples were positive. 15 Dot-ELISA negative samples were positive with IFA. Results showed agreement rate among both tests 90% ($P<0.000001$ and Kappa coefficient= 0.73). Dot-ELISA was configured to detect antibodies or parasite antigen in either micro titer plates for large-batch testing or with dip sticks for small numbers of determinations. A slight modification of the Dot-ELISA procedure allowed the determination of infection rates of vectors such as ticks and sandflies with parasites (Mehrabani *et al*, 2012).

The present results agreed with those of Mohammad *et al.* (2005) who evaluated sandwich and dot-ELISA for detection of CAg in the serum using anti-echinococcal hyperimmune rabbit sera and reported that, the sensitivity for the sandwich ELISA and dot-ELISA was 92.22% and 100%, respectively. Results showed that dot-ELISA was relatively better than ELISA. The high sensitivity of dot-ELISA was due to that nitrocellulose paper detected even trace amounts of antigen.

Chen *et al.* (2014) showed that this Dot-ELISA assay had both high sensitivity (92.9-97.6%) & specificity (95.2-98.4%) to detect *T. pisiformis* larval infections, and found very low levels of cross-reaction with other parasites. Wang *et al.* (2002) showed that dot-ELISA was strongly positive in serologically confirmed patients; the specificity of test was 98.75%. Dot-ELISA is rapid and important to confirm clinical diagnosis either in the laboratory or in the field (Hadighi *et al*, 2003). Dot-ELISA using pAbs used for detection of a hydatid antigen in serum for diagnosis of CE showed a sensitivity of 53.33% and specificity of 96.66%, whereas EITB gave a sensitivity of 46.66%. So, Dot-ELISA is a simple procedure for diagnostic detection of the hydatid antigen (Swarna and Parija, 2012).

Sedaghat *et al.* (2011) indicated that, a sensitivity of Dot-ELISA system 100% and specificity of 89.1% for CAg detection. The sensitivity of Dot-ELISA using cyst wall, protoscolex and cyst fluid was 96.66%, 86.66% and 93.33%, respectively and the specificity of the assay was 70% for dot-ELISA using cyst fluid, protoscolex and cyst wall antigens (Swarna and Parija, 2008). Dalimi-Asl *et al.* (2000) reported similar to results of the present study and of other authors that the Dot-ELISA was very sensitive and specific in detecting CAg of *E. granulosus*. In Dot-ELISA for detecting crude or purified antigens can be used. The crude antigen had a sensitivity of 97% and a specificity of 52% with ELISA. In previous study authors reported that, sensitivity for ELISA and Dot-ELISA was 92.22% and 100% respectively and specificity was 98.75% for both tests (Soliman *et al*, 2014).

Besides, Zhang and McManus (1996) used crude antigen of hydatid fluid for Dot-ELISA and reported a sensitivity of 92.3% and specificity of 89.9% for this test. In addition, the present study indicate high positive predictive value for dot-ELISA in diagnosis of human hydatid cyst infection that agree with some authors (Dalimi-Asl *et al*, 2000), thus this test was recommended for field study and community screening. Dot-ELISA was configured to detect antibodies or parasite antigen in either micro titer plates for large-batch testing or with dip sticks for small numbers of determinations. A slight modification of the Dot-ELISA procedure allowed determination of parasitic infection rates in vectors as ticks, mosquitoes and sandflies (Mehrabani *et al*, 2012).

Generally, cystic hydatidosis was found in Africa, Europe, Asia, the Middle East, Central and South America, but rare in North America. Infected dogs shed eggs in feces which contaminate the soil. Sheep, cattle, goats, and pigs ingest the eggs, which hatch and develop into cysts in the internal organs. Commonest mode of transmission to man is by accidental consumption of soil, water, or food contaminated by feces of an infected dog. *Echinococcus* eggs in soil can stay viable for up to a year. Hydatidosis infects all body organs and may be fatal, resulted in annual economic losses of several billion dollars in livestock sector due to low performance, morbidity and/or mortality of infected animals, and condemnation of infected organs of slaughtered ones (CDC, 2013).

In Egypt, endemic zoonotic hydatidosis were reported in man (El Shazly *et al*, 2007 a), farm animals (Hassanain *et al*, 2016) and equines (Haridy *et al*, 2008a). Natural *E. granulosus* infection was found in the street dogs (El Shazly *et al*, 2007b) and even pet ones or indoors as a zoonotic silent health problem (Haridy *et al*, 2008b).

## Conclusion

The antigen detection assay is superior and more sensitive than antibody detection assay specially in diagnosing active infection where hydatid cysts are predominant. Antigen detection assay might be a useful approach for assessment of the efficacy of treatment especially after removal of cyst.

Further studies are ongoing to improve the diagnostic efficacy of the antigen based ELISA method. Sandwich ELISA and dot-ELISA techniques appear proved useful for the detection of human echinococcosis, as well as in the edible animals Although both tests gave more or less similar results, the dot-ELISA was more acceptable with respect to its higher sensitivity, simplicity in practice and commercial availability.

Table 1: Reactivity of purified protoscoleces antigen by indirect ELISA

| Serum samples | (X±SD) |
|---|---|
| *Echinococcus granulosus* | 1.21± 0.13 |
| *Fasciola gigantica* | 0.19± 0.01 |
| *Toxoplasma gondii* | 0.27± 0.04 |
| *Cryptosporidium parvum* | 0.18± 0.03 |

OD: optical density; SD: standard deviation

Table 2: Reactivity of rabbit anti- *E. granulosus* IgG pAb against other parasitic antigens by indirect ELISA (OD reading = 492 nm)

| Parasitic antigen | (X ±SD) |
|---|---|
| *Echinococcus granulosus* | 1.94± 0.05 |
| *Fasciola gigantica* | 0.23 ±0.02 |
| *Toxoplasma gondii* | 0.14± 0.01 |
| *Cryptosporidium parvum* | 0.16 ± 0.11 |

Table 3: Sensitivity of Dot-ELISA techniques in detection of *E. granulosus* antigens in human.

| Groups | Score of the colour range |
|---|---|
| Healthy control (n=20) | - |
| *Echinococcus granulosus* (n=50) | ++++ |
| Other parasites (n=30) | |
| *Fasciola gigantica* (n=15) | +++ |
| *Schistosoma mansoni* (n=10) | ++ |
| Hookworm (n=5) | + |

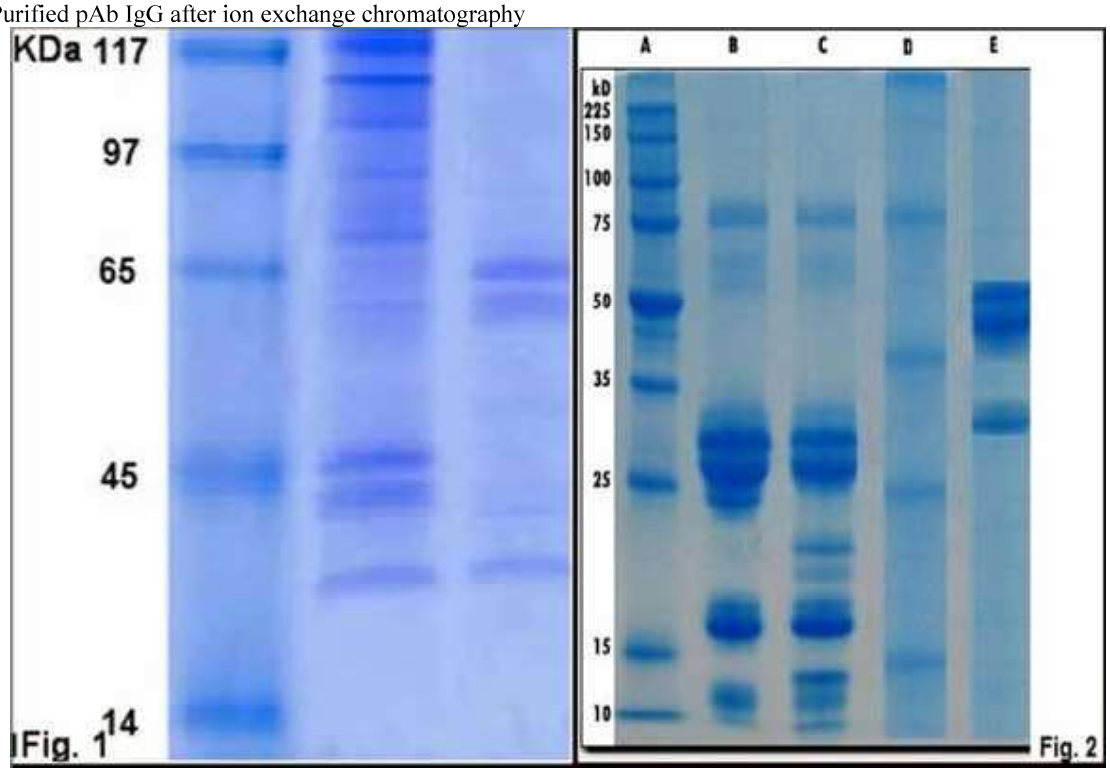
Table 4: Sensitivity, specificity, PPV & NPV % of Dot-ELISA to detect protoscoleces antigen in sera

| Dot-ELISA | %Sensitivity | %Specificity | %PPV | %NPV |
|---|---|---|---|---|
| | 96% | 94% | 94% | 90% |

## Explanation of figures

Fig. 1: 12.5% SDS-PAGE of protoscoleces antigen before and after purification. Lane 1: Molecular weight of standard protein. Lane 2: Crude protoscoleces antigen. Lane 3: Precipitated proteins after 12.5% SDS-PAGE.

Fig. 2: 12.5% SDS-PAGE of anti- *E. granulosus* IgG pAb before and after purification. Lane A: Molecular weight of standard protein. Lane B: Anti- *E. granulosus* IgG pAb before purification. Lane C: Purified pAb IgG after 50% ammonium sulfate treatment. Lane D: Purified pAb IgG after 7% caprylic acid treatment. Lane E: Purified pAb IgG after ion exchange chromatography




## References

**Biffin, AH, Jones, MA, Palmeri, SR, 1993:** Human hydatid disease: Evaluation of ELISA for diagnosis, population screening and monitoring of control programs. J. Med. Microbiol. 39: 48-52.

**Boctor, FN, Peter-Stek, JB, Kamal, R, 1987:** Simplification and standardization of Dot-

ELISA for human schistosomiasis, J. Parasitol. 73:589-92.

**Bradford, MM, 1976:** A rapid and sensitive method for the quantitation of micro-gram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-54.

**Brunetti, E, Junghanss, T, 2009:** Update on cystic hydatid disease. Curr. Opin. Infect. Dis. 22:497-502.

**Brunetti, E, Kern, P, Vuitton, DA, WHO/ WGE, WP, 2010:** Expert consensus for the dia- gnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 114:1-16.

**Burgu, A, Doğanay, A, Gönenç, B, Sarı-meh-metoğlu, HO, Kalınbacak, F, 2000:** Analysis of fluids of hydatid cysts from sheep by SDS–PAGE and determination of specific antigens in protein structure by western blot-ting. Turk. J. Vet. Anim. Sci. 24:493-500.

**CDC, 2013:** Echinococcosis: www.cdc.gov/ parasites/echinococcosis/

**Chaya, DR, Parija, SC, 2013:** Evaluation of newly designed sandwich enzyme linked im-munosorbent assay for the detection of hydatid antigen in serum, urine and cyst fluid. Trop. Parasitol. 3:125-31.

**Chen, L, Yang, D, Gu, X, Peng, X, Yang, G, 2014:** Evaluation of a novel Dot-ELISA assay utilizing a recombinant protein for the effective diagnosis of *Taenia pisiformis* larval infec-tions. Vet. Parasitol. 204:214-20.

**Craig, PS, Rogan, MT, Campos-Ponce, M, 2003:** Echinococcosis: disease, detection and transmission. Parasitol. 127: S5-20.

**Dalimi-Asl, A, Madani, R, Ghorbankha-ni, D, Salami, S, 2000:** Comparative evaluation of serodiagnostic techniques in cystic hydatid disease. Arch. SID. Arch. Razi Ins. 51.

**Eckert, J, Deplazes, P, Kern, P, 2011:** Alveo-lar Echinococcosis (*Echinococcus multiloculа-ris*) and other Forms of Echinococcosis (*Echi-nococcus vogeli* and *Echinococcus oligar-thrus*). In: Zoonoses, Brown, D, SR, Palmer, PR, Torgerson and EJL, Soulsby (Eds.). Ox-ford University Press, Oxford.

**El Amir, A, Rabia, I, Diab, MT, 2012:** Diag-nostic potential of purified protoscolex antigen for diagnosis of hydatidosis in naturally infect-ed sheep and human. Korian J. Parasitol. 11: 10-9.

**El Shazly AM, Awad SE, Hegazy MA, Moh-ammad KA, Morsy TA, 2007a:** Echinococ-cosis granulosus/hydatidosis an endemic zoon-otic disease in Egypt. J. Egypt. Soc. Parasitol. 37, 2: 609-22

**El Shazly, AM, Awad, SE, Abdel Tawab, A H, Haridy, FM, Morsy, TA, 2007b:** Echino-coccosis (zoonotic hydatidosis) in street dogs in urban and rural areas, Dakahlia Governo-rate, Egypt. J. Egypt. Soc. Parasitol. 37, 1: 287-98.

**Engels, D, Chitsulo, L, Montresor, A, Sa-violi, L, 2002:** The global epidemiological sit-uation of schistosomiasis and new approaches to control and research. Acta Trop. 82:139-46.

**Engvall, E, Perlman, P, 1971:** Enzyme linked immunosorbent assay (ELISA): Quantitative as-say of characterization G. J. Immunochem. 8: 871-4.

**Feng, X, Wen, H, Zhang, Z, Chen, X, Ma, X, *et al*, 2010:** Dot immunogold filtration assay (DIGFA) with multiple native antigens for rap-id serodiagnosis of human cystic and alveolar echinococcosis. Acta Trop. 113:114-20.

**Golassa, L, Abebe, T, Hailu, A, 2011:** Ev-aluation of crude hydatid cyst fluid antigens for the serological diagnosis of hydatidosis in cattle. J. Helminthol. 85:100-8.

**Hadighi, R, Dalimi, A, Madani, R, 2003:** Partially purified fraction (PPF) antigen from adult *Fasciola gigantica* for the serodiagnosis of human fascioliasis using Dot-ELISA tech-nique. Ann. Saudi Med. 24:18-20.

**Haridy, FM, Abdel Gawad, AG, Ibrahim, BB, Hassan, AA, El-Sherbi, GT, *et al*, 2008a:** Zoonotic hydatidosis in donkeys: post-mortum examination in the Zoo, Giza, Egypt. J. Egypt. Soc. Parasitol. 38, 1:305-12

**Haridy, FM, Holw, SA, Hassan, AA, Morsy, TA, 2008b:** Cystic hydatidosis: a zoonotic si-lent health problem. J. Egypt. Soc. Parasitol. 38, 2: 635-44.

**Hassanain, MA, Shaapan, RM, Khalil FA, 2016:** Sero-epidemiological value of some hy-datid cyst antigen in diagnosis of human cystic echinococcosis. J. Parasit. Dis. 40, 1:52-6.

**Hernandez, A, Cardozo, G, Dematteis, S, 2005:** Cystic echinococcosis: analysis of the serological profile related to the risk factors in individuals without ultrasound liver chan-ges living in an endemic area of Tacuarembo, Uru-guay. Parasitol. 130:455-60.

**Hidalgo, C, García, MP, Stoore, C, Ramírez, JP, Monteiro, KM, *et al*, 2016:** Proteomics analysis of *Echinococcus granulosus* protoscolex stage. Vet. Parasitol. 218:43-5.

**Izol Volkan, Eken, A, Aridogan, A, Kolt-as, S, Tansug, Z, 2012:** Acute urinary retention due to cystic echinococcosis: A case report. Can Urol. Assoc. J. 6:E192-4.

**Kamel, D, Farid, A, Ali, E, Rabia, I, Hendawy, M, El Amir, AM, 2013:** Diagnostic potential of target giardia lamblia specific antigen for detection of human giardiasis using coproantigen Sandwich ELISA. Wld. J. Med. Sci. 9:113-122.

**Kanwar, JR, Vinayak, VK, 1992:** The significance of free and immunecomplexed hydatid specific antigen(s) as an immunodiagnosis tool for human hydatidosis. J. Med. Microbiol. 37: 396-403.

**Koura, EA, Rabee, I, Ahmed, RO, Mohamed, WA, 2015:** Using of paramagnetic nanoparticles with immunomagnetic bead ELISA in dia- gnosis of hydatidosis (*Echinococcus granulo-sus*). Wld. J. Pharm. Sci. 3:2372-9.

**Lier, T, Simonsen, GS, Wang, T, Lu, D, Hau-kland, HH, *et al*, 2009:** Real-time polymerase chain reaction for detection of low-intensity *Schistosoma japonicum* infections in China. Am. J. Trop. Med. Hyg. 81:428-32.

**Lipman, NS, Jackson, LR, Trudel, LJ, Garcia, FW, 2005:** Monoclonal versus polyclonal antibodies: distinguishing characterization, application and information resources. ILAR J. 46: 3-8.

**Liu, Q, Wang, ZD, Huang, SY, Zhu, XQ, 2015:** Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasit Vectors 8:292-8.

**Mckinney, MM, Parkinson, A, 1987:** A simple, non-chromatographic procedure to purify immunoglobulins from ascites fluid. J. Immunol. Meth. 96:271-8.

**Mehrabani, D, Malekpour, A, Zahedi, S, Deh- ghani, S, Tondro, G, 2012:** Comparison of Dot-ELISA with IFA test for diagnosis of human toxoplasmosis and seroepidemiological evaluation of the disease. Middle-East. J. Sci. Res. 11: 1530-5.

**Mohammad, RS, Heshmatollah, T, Kiumarth, R, Mohammad, RRD, Mehdi, A, 2005:** Comparison of Dot-ELISA and Sandwich ELISA diagnostic tests in detection of human hydatidosis. Iranian Biomed. J. 9: 91-4.

**Nakane, PK, Kawaoi, A, 1974:** Peroxidase-labeled antibody: A new method of conjugation. J. Histochem. Cytochem. 22:1084-91.

**Nasrieh, MA, Abdel-Hafez, SK, 2004:** *Echinococcus granulosus* in Jordan: Assessment of various antigenic preparations for use in the serodiagnosis of surgically confirmed cases using enzyme immune-assays and the indirect haem-agglutination test. Diag. Microbiol. Infect. Dis. 48:117-23.

**Nouir, NB, Nuñez, S, Gianinazzi, C, Gorcii, MC, Müller, NL, *et al*, 2008:** Assessment of *Echinococcus granulosus* somatic protoscolex anti-\gens for serological follow-up of young patients surgically treated for cystic echinococcosis. J. Clin. Microbiol. 46:1631-8.

**Nowotny, A, 1979:** Basic Exercises in Immunochemistry, Springer Verlag, Berlin, Heidelberg, New York.

**Oudni-M'rad, M, M'rad, S, Babba, H, 2015:** Molecular and Epidemiology Data on Cystic Echinococcosis in Tunisia, Current Topics in Echinococcosis, Dr. Alfonso Rodriguez-Morales (ed.), In:Tech, Doi: 10.5772/60891.

**Pagnozzi, D, Biosa, G, Addis, MF, Mastrandrea, S, Masala, G, Uzzau, S, 2014:** An easy and efficient method for native and immunoreactive *Echinococcus granulosus* antigen 5 enrichment from hydatid cyst fluid. PLoS One 9: e104962.

**Qaqish, AM, Nasrieh, MA, Al-Qaoud. KM, Craig, PS, Abdel-Hafez, SK, 2003:** Sero-prevalences of cystic echinococcosis and the associated risk factors, in rural-agricultural, Bedouin and semi-bedouin communities in Jordan. Ann. Trop. Med. Parasitol. 97: 511-20.

**Rady, AA, El Aswad, BDW, Masoud, B M, 2014:** Comparative evaluation of different diagnostic techniques using laminated layer antigen for serodiagnosis of human hydatidosis. Res.J. Parasitol. 9:41-54.

**Rafiei, A, Craig, PS, 2002:** The immunodiagnostic potential of protoscolex antigens in human cystic echinococcosis and the possible influence of parasite strain, Ann. Trop. Med. Parasitol. 96:383-9.

**Riesle, S, Garcia, MP, Hidalgo, C, Galanti, N, Saenz, L, *et al*, 2014:** Bovine IgG subclasses and fertility of *Echinococcus granulosus* hydatid cysts. Vet. Parasitol. 205: 125-33.

**Robijn, ML, Koeleman, CA, Wuhrer, M, Royle, L, Geyer, R, *et al*, 2007:** Targeted identification of a unique glycan epitope of

*Schistosoma mansoni* egg antigens using a diagnostic antibody. Mol. Biochem. Parasitol. 151:148-61,

**Rogan, MT, Craig, PS, 2002:** Immunological approaches for transmission and epidemiological studies in Cestode Zoonoses-the role of serology in human infection. In: Craig, P, Pawlowski, Z. (Eds.), Cestode Zoonoses: Echinococcosis and Cysticercosis. IOS Press, Amsterdam.

**Sambrook, J, Fritsch, EF, Maniatis, T, 1989:** Detection and analysis of proteins expressed from cloned genes. In: Molecular Cloning: A Laboratory Manual: Cold Spring Harbor Laboratory Press, New York, USA.

**Sedaghat, F, Sadjjadi, SM, Hosseini, SV, Kazemian, S, Sarkari, B, 2011:** Evaluation of a simple Dot-ELISA in comparison with countercurrent immunoelectrophoresis for diagnosis of human hydatidosis. Clin Lab 57:201-205.

**Sheehan, D, Gerald, RF, 1996:** Ion-exchange chromatography. Meth Mol Boil 59: 145-50.

**Simsek, S, Koroglu, E, 2004:** Evaluation of enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) for immunodiagnosis of hydatid diseases in sheep. Acta. Trop. 92:17-24.

**Siracusano, A, Delunardo, F, Teggi, A, Ortona, E, 2012:** Host-parasite relationship in cystic echinococcosis: An evolving story. Clin. Develop. Immunol. 2012:7-9.

**Soliman, MG, Farid, AA, Shalash, IR, El-Qasem, AAA, El-Amir, AM, 2014:** Evaluation of Sandwich ELISA with Dot-ELISA as an immunodiagnostic assay for cystic hydatosis using *E. granulosus* protoscoleces antigens. Glob. Vet. 13:150-8.

**Sulbarán, GS, Ballen, DE, Bermúdez, H, Lorenzo, M, Noya, O, *et al*, 2010:** Detection of the Sm31 antigen in sera of *Schistosoma mansoni*-infected patients from a low endemic area. Parasite Immunol. 32:20-8.

**Sunita, T, Khurana, S, Malla, N, Dubey, M L, 2011:** Immunodiagnosis of cystic echinococc-osis by antigen detection in serum, urine, and sa-liva samples. Trop. Parasitol. 1:33-8.

**Swarna, SR, Parija, SC, 2008:** Dot-ELISA for evaluation of hydatid cyst wall, protoscoleces and hydatid cyst fluid antigens in the serodiagnosis of cystic echinococcosis. Rev. Inst. Med. Trop. Sao Paulo. 50:233-6.

**Swarna, SR, Parija, SC, 2012:** Evaluation of Dot-ELISA and enzyme-linked immuno-electro-transfer blot assays for detection of a urinary hydatid antigen in the diagnosis of cystic echinococcosis. Trop. Parasitol. 2:38-44.

**Tabar, GH, Haghparast, A, Borji, H, 2012:** Serodiagnosis of sheep hydatidosis with hydatid fluid, protoscolex, and whole body of *Echinococcus granulosus* antigens. Comp. Clin. Pathol. 21:429-32.

**Tijssen, P, Kurstak, E, 1984:** Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-anti-body conjugate for enzyme immunoassay. Anal. Biochem. 136: 451-7.

**Van Dam, GJ, Wichers, JH, Ferreira, T M F, Ghati, D, Van Amerongen, A, *et al*, 2004:** Diagnosis of schistosomiasis by reagent strip test for detection of circulating ca-thodic antigen. J. Clin. Microbiol. 42:5458-61.

**Wang, Y, Bradshaw, H, Rogan, MT, Craig, PS, 2002:** Rapid dot-ELISA for detection of specific antigens in the cyst fluid from human cases of cystic echinococcosis. Ann. Trop. Med. Parasitol. 96:691-4.

**Yamamoto, YI, Mineo, JR, Meneghisse, CS, Guimaraes, ACS, Kawarabayashi, M, 1998:** Detection in human sera to excreted secreted antigens *Toxoplasma gondii* by use immunoblot assay of IgG, IgM and IgA from of dot-ELISA. Ann. Trop. Med. Parasitol. 92:23-30.

**Yang, YB, Harrison, K, 1996:** Influence of column type and chromatographic conditions on the ion- exchange chromatography of immunoglobulins. Chromatography 743: 171-80.

**Zane, HD, 2001:** Laboratory safety and test quality assurance. In: Immunology: Theoretical and Practical Concepts in Laboratory Medicine; Saunders WB Company, Philadelphia, Pennsylvania, USA.

**Zhang, LH, McManus, DP, 1996:** Purification and N-terminal amino-acid sequencing of *Echinococcus granulosus* antigen 5. Parasite Immunology 18: 597-606.

**Zhang, W, Wen, H, Li, J, Lin, R, McManus, DP, 2012:** Immunology and immunodiagnosis of cystic echinococcosis: An update. Clin. Develop. Immunol. 12:10.

**Zhang, W, Zhang, Z, Shi, B, Li, J, You, H, Tulson, G, *et al*, 2006:** Vaccination of dogs against *Echinococcus granulosus* the cause of cystic hydatid disease in humans. J. Infect. Dis. 194: 966-74.