

Diagnosis of Echinococcosis (Hydatidosis) Using Dot-ELISA

Ramadan M. M.¹, El-Ameer A. M.², Shalash I. R.³, Taki-El-Deen F. M. A.¹, Abdeen G. S.¹

¹Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, ²Zoology Department, Faculty of Science, Cairo University, ³Theodore Bilharz Research Institute, Giza, Egypt

ABSTRACT:

Background: cystic echinococcosis (CE) is a complex, chronic and neglected disease caused by the larval stage of *Echinococcus granulosus*. The effects of this neglect have a powerful impact in remote rural areas whose population has no chances of being diagnosed and treated correctly without leaving their works and travelling long distances, sometimes taking days to reach the closest medical center. The present study was designed to evaluate the diagnostic efficacy of purified polyclonal antibody (PABs) raised against *Echinococcus granulosus* 50 and 31 kD proteins for detection of circulating hydatid antigen using dot ELISA. **Materials and methods:** the previous proteins from sheep and camel lungs was purified by ammonium sulfate and caprylic acid. The purified protein injected in Newzealand rabbits to raise specific polyclonal antibodies (pAb) against *E. granulosus*. Detection of 50 and 31 kD proteins in serum by dot-ELISA gave a sensitivity of 92.9%, a specificity of 95%. **Conclusion:** dot-ELISA techniques emerge to be adequately sensitive assays for the diagnosis of human echinococcosis using cathepsin B antigen.

Key words: Echinococcosis– 50 and 31 kd proteins – dot-ELISA technique.

INTRODUCTION

Human echinococcosis is a parasitic disease caused by tapeworms of the genus *Echinococcus* ⁽¹⁾. Echinococcosis, a severe zoonotic disease that may be fatal if untreated ⁽²⁾. Larval infection (hydatid disease, hydatidosis) is caused mainly by *Echinococcus granulosus* ⁽³⁾. Human cystic echinococcosis (CE) is a zoonosis caused by the larval stage of the *Echinococcus granulosus* and the most common sites affected are the liver and lung in approximately 80–90% of cases. The hydatid bone represents the 0.5–2.5% of all cases ⁽⁴⁾.

Hydatid cyst disease, accounting for over 95% of human echinococcosis, predominates in poor, pastoral communities that raise sheep and other livestock, and keep dogs for guarding and herding because of the complex two-host lifecycle ⁽⁵⁾.

Clinical diagnosis of CE is frequently difficult, hence always supported by imaging and immunological methods. The immunodiagnostic methods detecting the antibodies have the disadvantages of low specificity and sensitivity and the inability to differentiate between recent and past infections ⁽⁵⁾.

The diagnosis of hydatidosis is based on immunodiagnostic methods along with radiological and ultrasound examinations ^(6,7). Although various imaging techniques

such as ultrasonography or radiology easily detect CE in clinical settings, the primary diagnosis needs be confirmed by serological tests since the clinical signs of the disease are non-specific ⁽⁸⁾. A great number of immunological assays have been developed for detection of anti-hydatid cyst antibodies and recently, hydatid antigens in the serum ⁽⁹⁾.

Therefore, immunodiagnosis remains an important tool in the diagnosis of the disease. Chordi and Kagan⁽¹⁰⁾ were the first to use immunoelectrophoresis to identify the antigenic components of sheep hydatid cyst fluid (HCF) and subsequently determined which antigenic components were active in detecting antibodies in the sera of patients with hydatid cysts. A successful immunodiagnostic test depends on the use of highly specific and sensitive antigens, as well as the detection of the appropriate antibody class or subclass ^{(11), (12)}.

However, ELISA results showed highly variable sensitivities, and no cross-reactivity with other parasite species has been frequently reported ⁽¹³⁾.

The presence of a cyst-like mass in a person with a history of exposure to sheepdogs in areas where *E. granulosus* is endemic supports the diagnosis of cystic echinococcosis. However, echinococcal cysts must be differentiated from benign cysts, cavitary tuberculosis, mycoses, abscesses,

and benign or malignant neoplasms. A non-invasive confirmation of the diagnosis can usually be accomplished with the combined use of radiologic imaging and immunodiagnostic techniques. Radiography permits detection of echinococcal cysts in the lungs; in other sites, however, calcification is necessary for radiographic visualization. Computed tomography, magnetic resonance imaging, and ultrasonography are useful for diagnosis of deep-seated lesions in all organs and also for determination of the extent and condition of the avascular fluid-filled cysts. Abdominal ultrasonography has emerged as the most widely used imaging technique for echinococcosis because of its widespread availability and usefulness for defining number, site, dimensions, and vitality of cysts. 10 Portable ultrasonography machines have been applied for field surveys with excellent results ^{(14),(15)}. A standardized classification system for hepatic cysts detected by ultrasonography has been developed by the World Health Organization ⁽¹⁾.

Serology has been one of the methods selected for the post-operative control of hydatidosis. However, the long persistence of anti-*E. granulosus* antibodies after recovery hampers the diagnosis of relapse by serology. Antibody assays are useful to confirm presumptive radiologic diagnoses, although some patients with cystic echinococcosis do not demonstrate a detectable immune response ⁽¹⁶⁾. Hepatic cysts are more likely to elicit an immune response than pulmonary cysts. Regardless of location, the sensitivity of serologic tests is inversely related to the degree of sequestration of the echinococcal antigens inside cysts; for example, healthy, intact cysts can elicit a minimally detectable response, whereas previously ruptured or leaking cysts are associated with strong responses. The indirect hemagglutination test is sensitive but has now been replaced by the enzyme immunoassay (ELISA) for initial screening of sera. Specific confirmation of reactivity can be obtained by demonstration of specific echinococcal antigens by immunoblot assays. Eosinophilia is present in <25% of infected persons ⁽¹⁴⁾.

To overcome this several immunological tests, depending on antigen detection, have been developed as an alternative for echinococcosis diagnosis ⁽¹⁷⁾.

Detection of parasite antigen also helps to demonstrate the effect of treatment, and has a high specificity ⁽¹⁸⁾.

The present study was conducted to evaluate the diagnostic efficacy of purified polyclonal antibody (PAbs) raised against *Echinococcus granulosus* 31 and 50 kD proteins for detection of circulating hydatid antigen using dot-ELISA.

MATERIALS AND METHODS

Animal: Two New Zealand white male rabbits, weighting approximately 1.5 Kg and about 2 months age, purchased from rabbit research unit (RRU), Agriculture Faculty, Cairo University. They were examined before the start of the experiments and were used in the production of the antibodies ⁽¹⁹⁾. They were housed in the animal house in Theodore Bilharz Research Institute (TBRI), Giza, Egypt. They were kept for 4 weeks (experiment duration) under standard laboratory care at 21°C, 16% moisture, filtered drinking water with additional salt (1cm/5 liter) and vitamin (1cm/10 liter). Diet contains 15% protein, 3% fat and 22% fiber purchased from RRU. Animal experiments were carried out according to the Internationally Valid Guidelines.

Parasite: Hydatid cysts were removed from sheep and camel liver and lungs from an abattoir in Cairo Governorate and were transferred to our laboratory in TBRI in Hanks' buffer (Hanks' Balanced Salt Solution) (HBSS) to stimulate normal ion concentration under physiological tissue conditions ⁽²⁰⁾.

Sera samples: One hundred and thirty-two animals (sheep and camels) samples were collected during several visits to local abattoir (92 animals were infected by *E. granulosus* worms, 25 animals were positive for other parasites than *E. granulosus* and 35 animals were healthy control).

Sera were collected during slaughtering. The livers and lungs of animals undertaken in the study were checked for the adult flukes. Sera was allowed to clot at room temperature for 2 hr. Sera were separated by centrifugation at 2000 g for 10 min. and then fractionated into small aliquots and stored at -30°C until used.

Preparation of parasite antigen: *Echinococcus granulosus* hydatid fluid was collected from ovine fertile cysts for subsequent use as a specific parasite antigen and clarified by centrifugation at 10,000 g at

4°C for 60 min., dialyzed against phosphate buffer saline (PBS) pH 7.2. Protoscolecemes were prepared following the method of ⁽²¹⁾. In brief, protoscolecemes were collected and the viability was determined by the vital coloration approach with 0.2% eosin staining. The protoscolecemes were subjected to three cycles of freezing and thawing and suspension in 10 times their volume of 0.15 M PBS, pH 7.2. Subsequently, the protoscolecemes were suspended in 4 times their volume of PBS containing 0.1 mg aprotinin/ mL, then sonicated on ice in a 150 W ultrasonic disintegrator, until no intact protoscolecemes were visible microscopically and the supernatant solution was split into aliquots and stored at -20°C until further processing.

Purification and characterization of parasite antigen: Ammonium Sulfate, DEAE- Sepharose CL-6B and gel filtration chromatography on Sephadex G-25 column, these techniques for purification of antigen by separating proteins on the basis of charge and molecular size according to Smith, *et al* ⁽²²⁾. Then protein content was estimated by a Bio-Rad protein assay as shown by Bradford ⁽²³⁾. Finally characterization of protoscolex antigen by SDS-PAGE according to Laemmli ⁽²⁴⁾.

Production and Purification of Polyclonal Antibodies:

Before immunization, rabbits were assayed by ELISA for hydatid Abs and cross reactivity with other parasites. Rabbits were injected intramuscularly (i.m.) at four sites according to Fagbemi, *et al* ⁽²⁵⁾. With 100µg of purified protoscolex mixed with equal volume of complete Freund's adjuvant (CFA, Pierce, Rockford, IL, USA). Then, 3 booster doses (0.5 mg of purified protoscolex with equal vol. of incomplete Freund's adjuvant (IFA, Pierce) were given at one week intervals. One week after the last booster dose, the rabbit's sera were obtained and pAb fraction was purified by 50% ammonium sulfate precipitation method. More purification of pAb was performed by 4% caprylic acid method. The reactivity of anti-protoscolex antigen IgGpAb against *Echinococcus* antigens was assessed using indirect ELISA.

Diagnosis of Echinococcosis in Animal Sera by dot ELISA:

Dot-ELISA was performed according to McKinney and Parkinson ⁽²⁶⁾. The prewetted nitrocellulose membrane (NC) was transferred

to the Bio-Dot apparatus and washed once with 0.6 carbonate coating buffer for 5 min. After removing the excess solution, by suction, the NC coated with **10 µl of purified pAb IgG** diluted (1/500) in carbonate buffer. The strips were air-dried for 30 min and washed with PBS/T three times. Then it is blocked by 10 ul PBS 7.2 containing 2% BSA by incubating for 3 h at room temperature. Wash the strips with PBS/T three times and **100 µl of 1:10 dilution of positive and negative sera** was added and incubated for 1.5 h at 37°C with constant shaking. The strips were washed with PBS/T three times and incubated with pAb IgG conjugated to HRP at a dilution of (1/250) and incubated for 15-45 min. at 37°C in dark with constant shaking. The NC membrane was removed from the Bio-Dot apparatus and washed 5 times with PBS/T each time, followed by 2 times washing with PBS only. Then NC membrane immersed in substrate solution (DAB). The reaction was stopped, just after development of color, with cold dist. H₂O. Also Dot-ELISA was made with sera of naturally infected animals with other parasites such as *Giardia spp*, *H. nana* and *Fasciola spp*.

Statistical analysis: The data are presented as mean ± standard deviation of mean (X ± SD). The mean values of each group were calculated from the mean values of individual patients. The mean groups were compared by analysis of variance ⁽²⁷⁾. This may be accomplished by changing the selection of the reference value (i.e. cut-off) for the particular test ⁽³³⁾.

- Sensitivity= (no. of true +ve cases/ no. of true +ve cases + no. of false -ve cases).
- Specificity= (no. of true -ve cases/ no. of true -ve cases + no. of false +ve cases).
- Positive predictive value (PPV) = (No. of true +ve cases/ no. of true +ve cases + no. of false +ve cases).
- Negative predictive value (NPV) = (No. of true -ve cases/ no. of true -ve cases + no. of false -ve cases).

RESULTS

Calculation of Total Protein Content of The Antigen: The antigen obtained from hydatid cyst fluid contains 11 mg/ml of total protein as measured by Bio-Rad protein assay while it was 5.3 mg/ml after precipitation.

Reactivity of antigen by Indirect

ELISA: Serum samples from human infected with *E. granulosus* gave a strong reaction against purified antigen with mean OD reading equal to 1.89 and no cross reactions were recorded with sera of animals or patients infected with other parasites e.g., *Giardia*, *F. gigantica* and *Hymenolepis nana* (Table 1).

Production and Purification of Polyclonal Antibodies: Purification using the 50% ammonium sulfate precipitation method⁽²⁷⁾, the protein content was 10 mg/ml, while following 7% caprylic acid⁽²⁶⁾ precipitation method the content dropped to 6.4 mg/ml.

Characterization and reactivity of anti-*E. granulosus* IgG pAb: The purity of IgG after each step of purification was assayed by 12.5% SDS-PAGE under reducing condition. The purified pAbIgG was represented by H- and L-chain bands at 50 and 31 kDa respectively. The pAb appears free from other proteins. Reactivity of anti-*E. granulosus* antibodies against protoscolex antigen and other parasitic antigens (*S. mansoni*, hookworms, *F. gigantica*) was determined by indirect ELISA. The produced anti-*E. granulosus* antibodies diluted in PBS buffer gave strong reactivity to protoscolex antigen. The OD readings at 492 nm for *E. granulosus* were 1.93 compared to 0.33, 0.35 and 0.35 for *Giardia*, *H. nana* and *Fasciola*, respectively (Table 2).

DISCUSSION

CE is a public health and economic problem, concerning both humans and animals, and needs an early and definite diagnosis⁽²⁸⁾. Cystic hydatid disease (CHD) is detectable clinically through various imaging techniques such as ultrasonography or radiology. The primary diagnosis must be confirmed by more specific testing, such as serological tests based on the discovery of antibodies against the organismal antigens in the patient's serum⁽⁸⁾. Ordinary serological tests such as immunoelectrophoresis, double diffusion in agar, or indirect hemagglutination are being replaced by more sensitive assay methods such as enzyme-linked immunosorbent assay (ELISA), immunoblot (IB), and indirect immunofluorescent antibody test (IFA)⁽²⁹⁾. ELISA is a high-sensitivity test that is strongly recommended

for the detection of specific antibodies in cystic human disease (CHD) cases^{(6), (30)}. Antigen detection assay in serum is generally performed by dot-ELISA⁽³¹⁾.

Most of the serological tests such as ELISA performed on patients' sera for detection of specific antibodies gave rise to variable results of sensitivity and specificity. The results of antibody detection by indirect ELISA, using different antigens, showed that the hydatid fluid was the most effective antigen of those assessed for detection of infection with hydatidosis.

The results of antibody detection by indirect ELISA showed that the hydatid fluid was the most effective antigen for detection of hydatidosis in sheep and camels when compared with excretory/secretory and somatic antigens of protoscolex.

Protoscolex antigen used for immunization of rabbit for preparation of rabbit anti-*E. granulosus* pAb. 1 mg of protoscolex antigen were given to each rabbit in entire course of immunization in the first dose [1 mg protoscolex antigen mixed 1:1 in Freund's complete adjuvant (Sigma)] and 0.5 mg emulsified in incomplete Freund's adjuvant 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⁽³²⁾.

The purification procedures followed in this study were satisfactory, for IgGpAb two purification methods undertaken, ammonium sulfate precipitation which showed that, most of albumin was removed from rabbit anti-*E. granulosus* IgGpAb, 7% caprylic acid according to Goding⁽³³⁾. The purity of IgGpAb was assayed by 12.5% SDS-PAGE. In the present study, reactivity of purified pAb demonstrated the reactivity of pAb as determined by indirect ELISA, gave a strong reactivity to protoscolex antigen. The purified pAb was further used as a primary capture to coat ELISA plates. The secondary capture of pAb was by conjugation with Horse-Radish Peroxidase enzyme (HRP), dot ELISA was adopted using a pair of pAbs against protoscolex antigen, anti-*E. granulosus* IgGpAb and peroxidase-conjugated IgG polyclonal antibodies.

The **Diagnostic accuracy** for positivity in dot-ELISA for protoscolex antigen was equal to 93.5 in serum.

On detection of *E. granulosus* circulating antigen by dot ELISA in animal serum samples, sensitivity equal 90.48% and specificity equal 91.3%. In healthy control serum samples, no positive results were obtained giving specificity 100%, but PPV and NPV were 97.5%, 86.4% respectively,

In conclusion, dot-ELISA technique appear to be promising sufficiently sensitive assay for the diagnosis of echinococcosis .

REFERENCES

- WHO (2016):** Fact sheet. Available from: <http://www.who.int/mediacentre/factsheets/fs377/en/index.html>
- Umhang G, Lahoreau A, Hormaz V, Boucher J, Guenon A, Montange D, Grenouillet F and Boue F(2016):** Surveillance and management of *Echinococcus multilocularis* in a wildlife park. *65(3):245–250.*
- Bothale KA, Kolhe H, Mahore SD and Wilkinson AR (2015):** Diagnosis of primary hydatid cyst of thigh by fine needle aspiration cytology. *Indian J. Med. Microbiol., 33(1):151-3.*
- Fiori R, Coco I, Nezzo M, Kabunda G, Umama GE, Emmanuele G, Fraioli MF and Simonetti G (2014):** Spinal hydatidosis relapse: A case report. *Case Reports in Orthopedics, 2014: 1-6.*
- Parija SC (1990):** Review of parasitic zoonoses. 1st ed. New Delhi: AITBS, Pp: 463.
- Parija SC (1998):** A review of some simple immunoassays in the serodiagnosis of cystic hydatid disease. *Acta.Trop.,70: 17-24.*
- Sadjjadi SM, Ardehali S, Noman-Pour B, Kumar V and Izadpanah A(2001):** Diagnosis of cystic echinococcosis: imaging or counter-current immuno-electrophoresis? *East Mediterr.Health J., 7: 907-910.*
- Grimm F, Maly FE, Lu J and Llano R (1998):** Analysis of specific immunoglobulin G subclass antibodies for serological diagnosis of Echinococcosis by a standard enzyme-linked immunosorbent assay. *Clin.Diag.Lab.Immunol., 5: 613–616.*
- Ortona E, Rigano SR, Buttari B, Delunardo F, Ioppolo S, Margutti P, Profumo E, Teggi A, Vaccari S and Siracusano A (2003):** An update on immunodiagnosis of cystic echinococcosis. *Acta.Trop.,85: 165-171.*
- Chordi A and Kagan IG (1965):** Identification and characterization of antigenic components of sheep hydatid cyst fluid by immunoelectrophoresis. *J.Parasitol.,51: 63–71.*
- Matossian RM, Anami SY, Salti I and Araj GF (1976):** Serum immunoglobulin levels in human hydatidosis. *Int.J.Parasitol.,6: 367–371.*
- Sbihi Y, Janssen D and Osuna A (1997):** Specific recognition of hydatid cyst antigens by serum IgG, IgE, and IgA using western blot. *J.Clin.Lab.Anal.,11: 154–157.*
- Jenkins DJ, Gasser RB, Zeyhle E, Romig T and Macpherson CNL (1990):** Assessment of a serological test for the detection of *Echinococcus granulosus* infection in dogs in Kenya. *Acta.Trop., 47: 245-248.*
- Moro P and Schantz PM (2009):** Echinococcosis: a review. *International Journal of Infectious Diseases, 13:125-133.*
- Zhang W, Li J, Lin R, Wen H and McManus DP (2012):** Immunology and Serological Diagnosis of Echinococcosis, *Serological Diagnosis of Certain Human, Animal and Plant Diseases. Dr. Moslih Al-Moslih (Ed.), InTech, Pp: 8-150.*
- Zhang W and McManus DP (2006):** Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol. Med. Microbiol., 47:24-41.*
- Parija, S.C.(1990):** Review of parasitic zoonoses. 1st ed. New Delhi: AITBS, Pp: 463.
- Lawn SD, Bligh J, Craig PS and Chiodini PL (2004):** Human cystic echinococcosis: evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. *Am.J.Trop.Med.Hyg.,70: 329-335.*
- Van Dam GJ, Wichers JH, Ferreira TMF, Ghati D, Van Amerongen A and Deelder AM (2004):** Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *J.Clin.Microbiol.,42: 5458-5461.*
- Abdel-Megeed KN and Abdel-Rahman EH(2004):** *Fasciolagigantica*: immunization of rabbits with proteins isolated from coproantigen. *J.Egypt.Soc.Parasitol.,34 (2) : 631-642.*
- Waizy H, Weizbauer A, Modrejewski C, Witte F, Windhagen H, Lucas A, Kieke M, Denkena B, Behrens P, Meyer-Lindenberg A, Bach F and Thorey F(2012):** In vitro corrosion of ZEK100 plates in Hank's balanced salt solution. *Biom.Eng.online, 11:1-14.*
- Rafiei A and 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: 383-389.*
- Smith AM, Dowd AJ, McGonigle S, Keegan P S, Brennan G, Trudgett A and Dalton JP (1993):** Purification of a cathepsin L-like proteinase secreted by adult *Fasciola hepatica*. *Mol.Biochem.Parasitol.,62(1): 1-8.*
- Bradford MM (1976):** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal.Biochem.,72: 248-254.*
- Laemmli UK (1970):** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227 (5259):680-685.*
- Fagbemi BO, Obarisiagbon IO and Mbuh JV(1995):** Detection of circulating antigen in sera

- of *Fasciolagigantica* infected cattle with antibodies reactive with a *Fasciola*-specific 88-kDa antigen. *Vet.Parasitol.*,**58**: 235-246.
26. **Mckinney MM and Parkinson A (1987)**: A simple, non-chromatographic procedure to purify immunoglobulins from ascites fluid. *J.Immunol.Meth.*,**96**: 271-278.
 27. **Nowotny A (1979)**: Basic Exercises in Immunochemistry, Springer Verlag., Berlin, Heidelberg, New York, :Pp:7-20.
 28. **Pagnozzi D, Addis MF, Biosa G, Roggio AM, Tedde V, Mariconti M, Tamarozzi F, Meroni V, Masu G, Masala G, Brunetti E and Uzzau S (2016)**: Diagnostic accuracy of antigen 5-based ELISAs for Human cystic echinococcosis. *Impact* DOI: 10.1371/journal.pntd.0004585 Article in PLoS Neglected Tropical Diseases
 29. **Virginio VG, Hernandez A, Rott MB, Monteiro KM, Zandonai AF, Nieto A and Zaha A (2003)**: A set of recombinant antigens from *Echinococcusgranulosus* with potential for use in the immunodiagnosis of human cystic hydatid disease. *Clin.Exp.Immunol.*,**132** : 309–315.
 30. **Paul M and Stefaniak J (2001)**: Comparison of the dot immunobinding assay and two enzyme-linked immunosorbent assay kits for the diagnosis of liver cystic echinococcosis. *HepatoL.Res.*,**21** : 14–26.
 31. **Aly I, Zalat R, El aswad BW, Moharm IM, Masoud BM and Diab T(2013)**: Novel nanomagnetic beads based-latex agglutination assay for rapid diagnosis of human schistosomiasishaematobium.
 32. **Tendler M, Almeida MSS, Pinto RM, Noronha D and Katz N (1991)**: New Zeland rabbit model: Resistance induced by infection followed by active immunization with protective antigens. *J.Parasitol.*,**77**: 138-141.
 33. **Goding JW (1986)**: Monoclonalantibodies: principles and practice. Academic press inc.,Orlando, Florida, Pp:276.

Tables

Table 1: Reactivity of purified protoscolex antigen by indirect ELISA.

Serum samples	OD at 492 nm \pm SD
<i>E. granulosus</i>	1.89 \pm 0.54
<i>Giardia</i>	0.29 \pm 0.24
<i>Hymenolepis nana</i>	0.29 \pm 0.25
<i>Fasciola</i>	0.31 \pm 0.25

OD= optical density;
SD= standard

deviation.

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

Serum samples	OD at 492 nm \pm SD
<i>E. granulosus</i>	1.93 \pm 0.520
<i>Giardia</i>	0.33 \pm 0.228
<i>Hymenolepis nana</i>	0.35 \pm 0.254
<i>Fasciola</i>	0.35 \pm 0.224

OD= optical density; SD= standard deviation.

Table 4: Detection of circulating echinococcal antigen in serum of naturally infected animals

Group	N	-	1+	2+	3+	4+	\wedge P
E. granulosus	92	6 (3.9%)	4 (2.6%)	33 (21.7%)	27 (17.8%)	22 (14.5%)	<0.001*
Giardia	8	7 (87.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Hymenolepis nana	8	7 (87.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Fasciola	9	8 (88.9%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Control	35	35 (100.0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

X= mean; SD= standard deviation.