

Application of a modified human enzyme-linked immunosorbent assay kit for diagnosis of hydatidosis in sheep

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Summary

Cystic hydatid disease (hydatidosis) is one of the most important zoonosis that is caused by the larval stage of *Echinococcus granulosus*. As its diagnosis by clinical symptoms alone is difficult and confusing, serologic diagnostic techniques are used to confirm the disease. These techniques can also be used for epidemiologic studies. The present study was performed with a commercial human enzyme-linked immunosorbent assay (ELISA) kit for the diagnosis of hydatidosis in sera collected from sheep with hydatidosis. Sera were collected from 68 cases of hydatidosis proven by inspection of hydatid-infested livers and lungs of the sheep slaughtered in Mashhad abattoir and also from 11 healthy cases. Sera samples were examined by ELISA kit. The results showed that out of 68 cases of hydatidosis in sheep, 67 samples had positive absorbance. Also from 11 healthy samples, 9 had negative absorbance value. The sensitivity and specificity of the test were 99 and 82%, respectively. Therefore, it can be concluded that it is possible to use human ELISA kit for the diagnosis of hydatidosis in sheep.

Key words: *Echinococcus granulosus*, Antibody, ELISA, Sheep

Introduction

Echinococcus granulosus is a major infection with worldwide distribution and variable geographical incidence (Craig *et al.*, 2003). Live animals have the potential to develop the disease and need to be monitored (Kittelberger *et al.*, 2002). Diagnosis of hydatidosis, besides imaging techniques (e.g., digital radiography, ultrasonography, computerized tomography and magnetic resonance imaging) and clinical findings, relies on serologic techniques (Hadighi *et al.*, 2003). For development of a comprehensive immunological assay, antigenic macromolecules from every stage of the infectious agent should be considered. Purified oncosphere proteins caused strong antibody response in experimentally-immunized sheep and a recombinant oncosphere protein EG95 conferred high degree of protection in sheep, when used as

a vaccine. Therefore, oncosphere protein antigen may be potentially useful in sero-diagnosis for the ruminant intermediate host of this parasite (Lightowers *et al.*, 1999). For primary sero-diagnosis and for support of clinical diagnosis of cystic echinococcosis, the selection of immunodiagnostic test involves consideration of the diagnostic operating characteristics of the technique and the purpose for which it will be used. The diagnostic sensitivity and specificity of the tests vary according to the nature and quality of the antigen and the methodologic sensitivity of the selected technology (Poretti *et al.*, 1999). The availability of immunological tests with the ability to detect the majority of ruminants infected with *E. granulosus* would be desirable for animal import monitoring and also in countries where control schemes for the disease are operating (Kittelberger *et al.*, 2002). Since *E. granulosus* is the most

prevalent causative agent of hydatidosis in ruminants, the attempts are being focused to detect patients with hydatidosis by serological tests (Ghorbanalinezhad *et al.*, 2001). The hydatid antigen-based serological tests include indirect haemagglutination (IHA), indirect immunofluorescence (IFA), immunoelectrophoresis, counter-current immunoelectrophoresis (CCIEP), radio-immunoassay (RIA) and mainly the enzyme-linked immunosorbent assay (ELISA) (Ortona *et al.*, 2000). The ELISA test has been mainly used for immunodiagnosis of hydatid disease (Gottstein, 1984; Wattal *et al.*, 1986). Many of these assays with higher sensitivity and specificity require sophisticated equipment and trained technicians. Therefore, there is a need for a simple and inexpensive immunoassay. The objective of this study was to use ELISA for immunodiagnosis of hydatidosis in sheep.

Materials and Methods

Collecting sera

Sixty-eight blood samples were collected from sheep with hydatidosis and also 11 healthy cases from Mashhad abattoir, northeastern Iran. The samples were centrifuged at $2000 \times g$ at room temperature for five min to separate sera in Parasitology Department, School of Veterinary Medicine, Ferdowsi University of Mashhad. Sera were stored at -20°C until used.

ELISA assay

The assay was performed by *E. granulosus* ELISA kit produced by Pishtaz Teb Co. in a 96-well microtitration plates. The wells had been coated with secreted antigens of *E. granulosus* with the protein concentrations of $2.5 \mu\text{g/ml}$ phosphate buffer. All the solutions were used at $300 \mu\text{l}$ per well. Sera samples were diluted (1:10) by phosphate-buffered saline (PBS) included 1% bovine serum albumin (BSA) with a pH of 7.4. One-hundred μl of sera was loaded into duplicate wells and incubated for one hr at room temperature. Positive and negative control sera were used into duplicate. The wells were washed five times with PBS-Tween 20 (0.05%). After the final washing,

the plate slapped vigorously, well down on a bench top which covered with paper towels. Then, $100 \mu\text{l}$ of HRP-labeled polyclonal antibodies against sheep IgG at a 1:2,000 dilution in PBS containing 1% BSA was loaded into all the wells and incubated for one hr at room temperature. The plate was washed as described above to remove the excess conjugate. For colour development, $100 \mu\text{l}$ of TMB was added to each well as a substrate and the reaction was terminated after 15 min by the addition of $100 \mu\text{l}$ of 1 M HCl solution to each well. The absorbance at 490 nm was monitored in ELISA reader.

Evaluation of the ELISA kit

To evaluate human ELISA kit for detection of hydatidosis in sheep, it needs to calculate sensitivity, specificity and diagnostic accuracy as described in Table 1.

Table 1: Comparison of ELISA test results with actual hydatidosis status in sheep

Infection	Tests	
	Positive (n=69)	Negative (n=10)
Present (n=68)	67	1
Absent (n=11)	2	9

Results

All of 68 samples in this study had hydatid cysts in their liver. The presence of antibody against *E. granulosus* in sera from the 68 samples was investigated by ELISA. One of 68 samples with hydatidosis had negative and 67 had positive reactions (Table 1). Also, among 11 healthy sera from sheep, two found positive (Table 1). All these translated to a sensitivity, specificity and diagnostic accuracy of 99, 82 and 96%, respectively.

Discussion

The immunology of hydatid disease has been divided conceptually into pre- and post-encystment phases, which are differentiated by the formation of the laminated layer around the hydatid cyst. This occurs between two and four weeks post-infection in the animal intermediate or human host following ingestion of the egg and release of the oncosphere (Eckert and Deplazes, 2004).

This has caused a constant search for increasingly sensitive techniques to detect merely low antibody levels. To achieve this, several immunological methods have been evaluated in recent years (Zarzosa *et al.*, 1999).

In our study, results showed that the sensitivity and specificity of ELISA test were 99 and 82%, respectively. So, human ELISA kit for the diagnosis of hydatidosis, may be also used for the diagnosis of sheep hydatidosis. A recent study by Kittelberger *et al.* (2002) evaluated three ELISA tests employing a purified antigen B subunit, a recombinant oncospherical antigen (EG95) and a crude protoscolex antigen in 249 natural or experimentally-infected sheep. The highest diagnostic sensitivity yielded was 63% with the protoscolex antigen, but specificities were as high as 96%. Although these sensitivities are relatively low in terms of individual cases, the assay could be beneficial if used on a flock basis.

In a study with a crude antigen of sheep for the detection of serum antibody in sheep infested with *E. granulosus* by ELISA, its diagnostic sensitivity and specificity were up to 62.7 and 98.2%, respectively (Kittelberger *et al.*, 2002). In another study, for detection of human hydatidosis by antigens of a crude hydatid fluid from lung and liver of sheep, the sensitivity and specificity of ELISA were 99.22 and 98.75%, respectively (Siavashi *et al.*, 2005). Three tests including ELISA, CCIEP and IHA using crude and purified antigens compared with each other. Among these tests, ELISA had the lowest cross-reaction with sensitivity of 68% in all tests and crude antigen showed the highest sensitivity (Abdel and Hady, 1996). It has been reported that the sensitivity and specificity with antigens of hydatid cyst fluid of camel in an ELISA to measure total *E. granulosus* specific IgG antibody were 100 and 98.4%, respectively (Ramzy *et al.*, 1999). In another study, carried out for the diagnosis of hydatidosis in human by a new ELISA kit and using the sheep crude antigens, the sensitivity and specificity of the ELISA test were 93.3 and 96.6%, respectively (Ghorbanalinezhad *et al.*, 2001). Lower specificity of the ELISA reported in this study could be due to the poor quality enzyme conjugated anti-im-

munoglobulin. It has been shown that sheep produce specific immunological response during natural *E. granulosus* infections (Xiao *et al.*, 2003). However, it seems that these responses do not lead to raised serum antibody level in many animal or they are not maintained throughout the course of the infection (Kittelberger *et al.*, 2002).

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