Archives of Razi Institute, Vol. 62, No. 2, Spring (2007) 101-104

Short Communication

A serological survey for hydatidosis among buffaloes in Orumia

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Received 25 Jun 2006; accepted 08 Feb 2007

ABSTRACT

Hydatid cyst is an important and zoonotic infection caused by cystic stage of *Echinococcus granulosus.* It is a major and economic problem in most areas of the world that livestock are kept such as Iran. In this study, a total of 111 buffalo's sera from Center of Buffalo Sperm Preparation (CBSP), Orumia (West Azarbayjan province, Iran, 2003) were examined for the presence of hydatidosis by using enzyme-linked immunosorbent assay (ELISA). 5 µg protein per mL of antigen B from sheep hydatid cyst fluid was used in this assay. Optimum dilution for rabbit anti-bovine peroxidase conjugate (Sigma) was used 1:5000. Overall results indicated 32.4% of serum samples positive for hydatidosis. Results indicate buffalo is sensitive intermediate host for *Echinococcus granulosus* in this province.

Keywords: Antigen B, Hydatidosis, Buffalo, ELISA, Orumia

INTRODUCTION

Hydatid disease is a parasitic infection caused by cestodes of the genus *Echinococcus.* The most important and widespread of these parasites is *E. granulosus*, which causes cystic hydatid disease. The lifecycle involves two mammalian hosts. Dogs and other canids are infected with the parasite in the small intestine and eggs are released with faeces. Ingestion of the eggs by a wide variety of herbivorous animals leads to the growth of hydatid cysts in tissues. When infected tissues are eaten by a dog, the lifecycle is completed. Control programs for hydatid disease have been, or are being, undertaken either nationally or in regional areas. These programs rely on public education, restrictions on livestock slaughtering and control measures in dogs. Despite substantial efforts to reduce transmission of the parasite, hydatid disease remains a serious cause of human morbidity in many parts of the world (Schantz *et al* 1995 and Gemmell *et al* 1987). Recently, a vaccine has been developed as a new tool to assist with control of hydatid disease in livestoke (Lightowlers *et al* 1999). Some sensitive and specific serological tests are useful for both epidemiological studies and control programmes. Recently by using major hydatid fluid antigens of *E.granulosus* (Williams *et al* 1971, Yong & Heath

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1984) principally antigen B, immunodiagnosis tests have been appeared be useful for diagnosis of animal hydatidosis, particulary in field studies. In this study, the prevalance of hydatidosis among buffaloes in Center of Buffalo Sperm Preparation (CBSP), Western Azarbyjan province, was studied.

MATERIALS AND METHODS

Specimens. From 120 buffaloes in CBSP, western Azarbayjan province, 111 blood samples were obtained at 2003. Buffaloes were different ages from 4 months to 14 years old. Positive pooled control sera were obtained from 3 experimentally infected buffaloes, 6 months after experimental infection. Negative pooled control sera were obtained from 3 young non-infected buffaloes at the time of necropsy. Known positive and negative controls were included in all the test plates.

Antigen B preparation. Antigen B was prepared from crude sheep fertile hydatid cyst fluids as described by Oriol *et al* (1971), Gillespie and Hawkey (1995) and Ibrahem *et al* (1996). Briefly 100 ml of hydatid fluid was centrifuged at 1000 g for 15 min, and supernate dialysed against 0.005 M acetate buffer (pH=5.0), overnight at 4 ºC. The fluid was centrifuged at 50000 g for 30 min , and the precipitate resuspended in 10 ml of 0.2 M PBS (pH=8.0). The solution was mixed with ammonium sulfate to 40% saturation and hold for 60 min, and then centrifuged to remove the resulting precipitated globulin fraction. The supernatant was kept in a boiling water-bath (100 ºC) for 15 min. The solution was centrifuged at 50000 g for 60 min and collected the resulting supernatant containing antigen B. The protein concentration was determined by Lowry method (1951).

ELISA. Polystyrene flat bottomed 96-well Maxisorb plates (NUNC, Denmark) were used for ELISA assay. Antigen B with pooled positive and negative sera were titrated, using a checkerboard titration method with 0.625 to 20 µg/ml Antigen B. Rabbit anti-bovine IgG peroxidase conjugate (whole molecule, Sigma) was titrated, using a checkerboard titration method with 1:500 to 1:20000 dilutions. Optical density (OD) reading of positive and negative sera in each case was evaluated by using signal to noise (S/N) ratio. The S/N ratio is the ratio of observed OD reading of positive serum to observed OD reading of negative serum, at the same dilution. Optimal antigen concentration (2.5 µg /ml), conjugate dilution (1:5000) and sera titer (1:200) were determined. ELISA assay was performed as described by Craig (1986). Plate wells were coated overnight at 4 ˚C with 0.1 ml of 2.5 µg/ml antigen B in carbonate-bicarbonate buffer (pH=9.6). Plates were washed with 0.2 ml/well washing buffer (0.01 M PBS with 0.05% Tween20, pH=7.2). A blocking solution (0.2 ml/well) containing 1% bovine serum albumin (BSA) prepared in washing buffer was added and incubated overnight at 4 ˚C. After washing, the sera were diluted 1:200 in dilution buffer (washing buffer containing 0.1% BSA) and added 0.1 ml/well in triplicate. Plate was incubated 1 hour at 37 ˚C. After washing, HRP-rabbit anti bovine IgG conjugate (diluted 1:5000 in dilution buffer) was added and incubated 1 hour at 37 ˚C. Ortho phenylen diamine as a substrate was used.

RESULTS ANA DISCUSSION

The ELISA cut off value (the mean OD values plus three standard deviations in sera from non-infected buffaloes) was 0.570. Out of 111 serum samples 36 cases (32.4%) showed positive reaction. An immunodiagnostic test as a screening tool to detect hydatidosis cases in sero-epidemiological studies should be based on its simplicity, high sensitivity and high specificity. Some hydatid cyst components have been identified for application in the sero diagnosis of the disease. The most predominant of this parasite proteins are antigen B and antigen arc 5 which both of them have diagnostic values (Lightowlers *et al* 1984, Farag *et al*

1975) reported a specificity and sensitivity of 96% and 95.2% respectively in ELISA based on antigen 880 (this antigen probably was enriched in heat-stable antigen B). Njeruh & Gathuma (1990) obtained 100% specificity and 91% sensitivity in ELISA for natural hydatidosis in sheep and goats. Ibrahem *et al* (1996) applied partially purified preparations of hydatid fluid antigen B and a recombinant antigen B product in an ELISA in naturally infected sheep. The native antigen B preparations from camel hydatid cyst fluide gave the highest sensitivity (total 90%) with 99% specificity in the ELISA .The recombinant antigen B was the least sensitive antigen (25%), although it was highly specific (99%). False positive reactions commonly occur in populations having a high level of parasitism with helminths and poor specificity and sensitivity being major problems when using crude hydatid cyst fluid antigen (Njeruh & Gathuma 1987). Various studies in Iran have indicated that hydatid cyst is commonly founded in sheep, camels, cattles and goats (Mobedi *et al* 1970, Eslami 1990, Oryan *et al* 1994, Mobedi & Dalimi 1994, Dalimi *et al* 2002). The prevalence of hydatid cyst in sheep, goats and cattles is respetively 22.97%, 7.19% and 33.83% at slaughterhous in Ilam province (Dalimi *et al* 2004). In Iran, this ratio in livestoke is from 5% to 60% in different provinces (Dalimi *et al* 2004). The prevalence of hydatid cyst in slaughtered boffaloes has been reported from 0.89% to 57.76% in different studies in Iran (Navidpour *et al* 2003). In our study, the prevalence of disease is higher than previous reports that is caused sensetivity of our test. There is no doubt some cross reactions would be occurred with other parasitic helminths in field studies (Yong & Heath 1984). There is probably 0.5 million boffalos in Iran that 25% of them are existed in west azarbayjan province (Mohsenpour 2000). Control programs for hydatid disease have been or are being, undertaken either nationally or in regional areas in some countries. These programs rely on public education, restrictions on livestock slaughtering and control measures in dogs. Despite of substantial efforts to reduce transmission of the parasite, hydatidosis remains an important disease in many parts of the world. Recently, a vaccine has been developed as a new tool to assist with control of hydatid disease (Lightowlers 1999).

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