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Antigen based detection of cystic echinococcosis in buffaloes using ELISA and Dot-EIA

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Abstract Cystic echinococcosis is caused by the larval stage of the dog tapeworm, *Echinococcus granulosus*. The disease is recognized as one of the world's major zoonoses affecting human beings and domestic animals apart from its economic and public health importance. Development of the cysts in the intermediate host such as buffaloes occurs in the lungs, liver and other organs. In this study, detection of circulating antigen in the diagnosis of cystic echinococcosis in buffaloes was done using enzyme linked immunosorbent assay and Dot-Enzyme immunoassay (Dot-EIA). The sensitivity and specificity were determined as 89 and 92 % respectively, whereas those of Dot-EIA were determined as 94 and 96 %.

Keywords Cystic echinococcosis · Buffaloes · Antigen detection · ELISA · Dot-EIA

Introduction

Cystic echinococcosis, a zoonotic disease of man and animals is caused by the larval stage (metacestodes) of the dog cestode, *Echinococcus granulosus*, the life cycle involving two mammalian hosts. Definitive hosts are dogs in whose intestine the adult worms occur. Intermediate hosts are herbivores and omnivores wherein the development of the cysts occurs in liver, lungs and other organs. Accidental infection of man occurs during natural transmission of the parasite between the canid definitive hosts and domestic livestock intermediate hosts. In the middle east, the prevalence of cystic echinococcosis is high in man (E[illegible]ntaseb 1984) and in sheep, goats, cattle and camels (Al Yaman et al. 1985). Irrespective of the host species infected, cysts occur mostly in liver and lungs with varying degrees of involvement of one or other of these organs (Schantz 1972). The disease remains asymptomatic in many cases and in livestock intermediate hosts like buffaloes, the cysts are seen only on post mortem. Immunodiagnostic tests for cystic echinococcosis rely on the detection of serum antibodies and vary in their sensitivity and specificity with false negatives (Craig and Rickard 1981). To overcome such problem, the detection of circulating antigen rather than antibodies could be very useful in the immunodiagnosis of cystic echinococcosis particularly if circulating antigen is present in the false negatives (Craig and Nelson 1984). Hence, in the present study, detection of antigen in the diagnosis of cystic echinococcosis in buffaloes using ELISA and Dot-EIA was carried out to find out the sensitivity and specificity of these two assays.

Materials and methods

Sera from buffaloes

Blood samples were collected from buffaloes at the time of slaughter at the rate of 10 ml blood per buffalo in 30 ml test tubes and allowed to clot. The blood samples were refrigerated overnight and the sera separated, centrifuged at 2000 rpm for 15 min. The clean sera was pipetted into sterile 5 ml plastic vials, preserved by adding merthiolate

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RETRACTED ARTICLE

solution to a final concentration of 1:10,000 and stored at −20 °C.

Preparation of hydatid fluid antigen (HFA)

Hydatid cysts were collected from sheep and buffaloes from the slaughter house at the time of slaughter. Cysts were brought to the laboratory immediately and were washed with sterile normal saline. The hydatid fluid from the clean cysts was aspirated using a 20 ml glass syringe, transferred into a glass container and allowed to stand for few hours. The hydatid fluid was examined microscopically to ascertain the presence (fertile) or absence (sterile) of scolices so as to assess the fertility status of the cysts. The supernatant fluid fluid from the fertile cyst was carefully aspirated and dialysed in dialysis membrane bag against Polyethylene glycol 6000 (Carbowax) for 1 h to concentrate the fluid to half of its original volume. The dialysed HFA was stored in 5 ml quantities in 10 ml sterile plastic screw cap vials with merthiolate at 1:10,000 concentration as preservative at −20 °C.

Estimation of protein content of the hydatid fluid antigen

The protein content of the dialysed hydatid fluid antigen was estimated by the method of Lowry et al. (1951).

Raising of hyperimmune serum

Three healthy rabbits were hyperimmunised with three intramuscular injections of 2 ml of dialysed hydatid fluid antigen with Freund's adjuvant (Sigma, USA) at an interval of 14 days. The first injection of DHF antigen was with Freund's Complete Adjuvant (FCA) and the second and third injections were given with Freund's Incomplete Adjuvant (FIA). The rabbits were bled by cardiac puncture 8–10 days after the third injection. About 5 ml of blood was collected from each rabbit and hyperimmune serum was separated, centrifuged at 3000 rpm for 15 min, preserved with merthiolate solution in 2 ml aliquots in 5 ml sterile plastic screw cap vials and stored frozen at −20 °C.

Enzyme linked immunosorbent assay (ELISA)

ELISA recommended by Judson et al. (1985) for detection of circulating antigen was followed with modifications. The polystyrene 96 well flat bottom plates were coated with hyperimmune serum raised in rabbits against dialyzed hydatid fluid antigen at a dilution of 1:100. Before addition of the test sera, the wells in the ELISA plate were blocked with 1 % Bovine serum albumin and incubated for 1 h at 37 °C. Test sera from buffaloes were used at a dilution of 1:100 in duplicate. The plates were incubated at 37 °C for 1 h. Known human anti-hydatid serum at a dilution of 1:100 was used to detect the circulating antigen. At the end of 1 h incubation, after each step, anti-human HRP conjugate was used at a dilution of 1:8000. Each plate had two HRP and one control well. The plates were incubated at 37 °C for 1 h. After the period of incubation the substrate solution (ABTS) prepared with sodium citrate buffer (pH 4.2) and hydrogen peroxide was immediately dispensed into each well in 100 µl volume using a octapipette. After addition of substrate, the plates were incubated at room temperature for 20-30 min for development of colour reaction. At the end of this period, 100 µl of hydrofluoric acid was added and the plates were read in an ELISA reader at 405 nm. In this assay, any absorbance value which was twice and above the absorbance value of the negative serum was considered as positive.

Dot-Enzyme immunoassay (Dot-EIA)

The assay was carried out as per the method of Romia et al. (1992) for detection of circulating hydatid antigens with certain modifications. Dot-Enzyme Immunoassay (Dot-EIA) of collected serum samples from buffaloes was performed using the hyperimmune serum raised in rabbits against dialysed hydatid fluid antigen. Nitrocellulose membrane (NCM) was cut into 5 × 10 cm sizes for testing the sera samples from buffaloes. Rough squares were made on the NCM with each square numbered and coated with hyperimmune serum at a dilution of 1:100 in 1 µl volume. The unsaturated sites were blocked by immersing the NCM in 5 % dried milk powder and incubated at 37 °C for 1 h. Test sera from buffaloes were used at 1:100 dilution and incubated for 1 h at 37 °C. Human antihydatid serum was used in 1 µl volume at 1:100 dilution. The NCM was then incubated in 1:8000 anti-human IgG-HRP conjugate for 1 h. Following this the substrate solution diaminobenzidine was added for development of reaction in positive samples. In this assay, an appearance of brown dot at the site of application of the sample was considered positive.

Results

In the present study, for detection of circulating antigen for cystic echinococcosis, blood samples from 200 buffaloes (85 samples from hydatid positive buffaloes confirmed on slaughter and 115 samples from buffaloes without visible hydatid cysts) were collected at the time of slaughter and the sera separated for use in ELISA and Dot-EIA. The assays were performed using hyperimmune serum raised in rabbits against dialysed hydatid fluid antigen.

A total of 64 hydatid cysts were collected from sheep. Out of these, 35 cysts were found to be fertile showing a fertile cyst percentage of 54.7 %.

The protein content of the dialysed fertile hydatid cyst fluid from sheep and buffaloes was found to be 14 and 9 mg/ml of dialysed fertile hydatid cyst fluid respectively. Gatne et al. (1990) estimated the protein content of hydatid fluid and observed the protein content ranged from 16 to 20 mg/ml of the fluid.

Seventy six buffaloes were detected positive out of 85 sera samples from hydatid positive buffaloes using hyperimmune serum against dialysed hydatid fluid antigen by ELISA. From 115 sera samples from buffaloes without any visible hydatid cysts, 9 sera samples were detected false positive. By ELISA, the sensitivity and specificity of the assay in detecting antigen for cystic echinococcosis were determined as 89 and 92 %, respectively.

By Dot-EIA, 80 of the 85 sera samples from buffaloes with hydatid cysts proved positive. Four sera samples from 115 buffaloes with no visible hydatid cysts were detected false positive using the hyperimmune serum against dialysed hydatid fluid antigen. A sensitivity of 94 % and a specificity of 96 % were observed in Dot-EIA in detecting antigen in the diagnosis of cystic echinococcosis.

Discussion

Serological tests have been reported to be very useful in antemortem diagnosis of cystic echinococcosis in food animals such as sheep, goats, cattle, buffaloes etc. The use of serological tests helps to diagnose the condition before death of the animal and diagnosis of cystic echinococcosis is usually done during post mortem. Use of hydatid cyst fluid as antigen in serological diagnosis depends mainly on the presence of protein in the fluid which should be above 5 mg/ml of hydatid cyst fluid. Gatne et al. (1990) estimated the protein content of hydatid fluid and observed the protein content ranged from 16 to 20 mg/ml of the fluid, whereas in the current study the protein content was 14 and 9 mg/ml of dialysed fertile hydatid cyst fluid respectively, in sheep and buffalo hydatid cysts.

Craig and Nelson (1984) utilized ELISA and reported the test to be [illegible] % sensitive, the results in the present study coincide with the earlier report. Similarly, Moosa and Abdel Hafez (1994) reported circulating antigen detection using ELISA, also decreased the number of false negatives and was more sensitive. The differences in the sensitivity and specificity of the assay may be attributed to low levels of circulating antigen/immune complexes in natural infection of false negative animals, whereas the false positivity could be due to cross reacting circulating antigens of other metacestodes.

Romia et al. (1992) reported circulating antigen detection to be 86 % sensitive by Dot-EIA and the low sensitivity was attributed to small amounts of circulating antigens and immune complex formation. The increased sensitivity and specificity as observed in the present study could be due to high levels of circulating antigens which in turn reduces the number of false negatives and false positives.

Dot-EIA, owing to its higher sensitivity and specificity in the present study can be performed for diagnosing cystic echinococcosis at field level because of its simple procedure and rapid result whereas ELISA requires high end equipments with laboratory procedures.

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References

Al Yaman FM, Assaf L, Hailat N, Abdel Hafez FH (1985) Prevalence of hydatidosis in slaughtered animals from North Jordan. Ann Trop Med Parasitol 79:501–506

Craig PS, Nelson GS (1984) The detection of circulating antigen in human hydatid disease. Ann Trop Med Parasitol 78:219–227

Craig PS, Rickard MD (1981) Studies on the specific immunodiagnosis of larval cestode infections of cattle and sheep using antigens purified by affinity chromatography in an enzyme linked immunosorbent assay (ELISA). Int J Parasitol 11:441–449

El-Muhtaseb HH (1984) Surgical management of hydatid cysts of the liver: retrospective study of 75 cases. Jordan Med J 18:35–46

Gatne ML, Narasapur VS, Deshpande VS, Niphadkar SM (1990) Protein content and electrophoretic patterns of hydatid fluid. Indian Vet J 67:169–170

Judson DG, Dixon JB, Clarkson MJ, Pritchard J (1985) Ovine hydatidosis: some immunological characteristics of the seronegative host. Parasitology 91:349–357

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein determination using Folin–Ciocalteu reagent. J Biol Chem 193:265–275

Moosa RA, Abdel Hafez SK (1994) Serodiagnosis and sero epidemiology of human unilocular hydatidosis in Jordan. Parasitol Res 80:664–671

Romia SA, Yousuf ME, Handoussa AE, Rizk HM, Sallam SM (1992) Dot-ELISA as a diagnostic test in hydatid disease. J Egypt Soc Parasitol 22:603–610

Schantz PM (1972) Hydatidosis: magnitude del problemyt perspectivase de control. Biol Sanit-Panam 74:187–197 [Schwabe, C.W.1986. Current issues of hydatid disease: a zoonosis of increasing importance in R.C.A. Thompson] The Biology of Echinococcus and hydatid disease. George Allen and Unwin, London, pp. 81–113.