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Serodiagnosis of hydatidosis in buffaloes by counter immuno electrophoresis (CIE) and enzyme linked immunosorbent assay (ELISA)

A SANGARAN[1] and LALITHA JOHN[2]

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 007 India

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Hydatidosis, a silent cyclozoonotic infection of domestic animals and human beings caused by the larvae of dog cestode *Echinococcus granulosus* (Eckert *et al.* 2001), has a worldwide distribution and is one of the most important zoonotic diseases prevalent in different parts of the world. Several serological assays were developed for detection of anti-hydatid cyst antibodies, which includes counter immuno electrophoresis (CIE) and enzyme linked immunosorbent assay (ELISA) (Sadjjadi *et al.* 2007).

Blood samples were collected from 100 buffaloes (75 buffaloes with known hydatid cysts confirmed on slaughter and 25 buffaloes without any visible hydatid cysts), brought to the laboratory, sera separated and stored at –20°C with merthiolate as preservative for use in serodiagnostic tests. Hydatid cysts were collected from buffaloes at the time of slaughter, brought to the laboratory and cleaned with sterile normal saline.

The hydatid cyst fluid was aspirated using a glass syringe, transferred to a glass container and allowed to stand for 2 h. The fertility and sterility of the hydatid cyst was ascertained by examining the hydatid cyst fluid based on the presence or absence of protoscolices in the cyst fluid.

The supernatant of the hydatid cyst fluid was carefully separated, dialysed against polyethylene glycol 6000 so as to concentrate the fluid to half of its original volume. The dialysed fluid was stored in 2 ml aliquots with merthiolate as preservative at –20°C for use as antigen in the serodiagnostic tests using CIE and ELISA.

The protein content of the dialysed hydatid fluid was estimated as per Lowry *et al*. (1951). Hyper immune sera was raised in rabbits using Freund's complete adjuant (FCA) and Freund's incomplete adjuant (FICA) to be used as positive control in CIE and ELISA. Foetal calf serum was used as negative control in both the tests.

Present address: [1] Associate Professor, Department of Veterinary Parasitology, [2]Dean, Madras Veterinary College (e mails: [1]sangaranvet@gmail.com, [2]lallijohn@yahoo.com).

Sera samples (100 : 75 sera from buffaloes with known hydatid cysts confirmed on slaughter and 25 sera from buffaloes without any visible hydatid cysts) were serologically screened using CIE and ELISA.

Counter immuno electrophoresis (CIE): The CIE was carried out as per Ardehali *et al*. (1977) with certain modifications using barbitone buffer pH 8.6. Sera samples (100) collected from buffaloes were used as test sera in CIE.

A current of 150 volts was passed for 45 – 60 min and the slides were observed for formation of precipitation line and then stained using Amido black stain.

Enzyme linked immunosorbent assay (ELISA): ELISA was performed as per Iacona *et al.* (1980) with modifications (Yong and Heath 1984). Hyper immune sera served as a positive control and foetal calf serum was used as a negative control.

The optimum concentration of dialysed antigen, test sera and anti-bovine IgG-horse radish peroxidase were standardized by a chequer board titration. The optimum concentration of dialysed hydatid fluid antigen, test sera and the conjugate used in the present work were 1: 200, 1: 100 and 1: 5000 respectively. In this assay, any absorbence value which was twice and above the absorbence value of the negative serum was considered positive. The absorbence values were recorded at 405nm in a multiscan ELISA reader.

Using CIE, out of 75 buffaloes with hydatid cysts, 52 were found positive and only 4 sera samples were found positive among the 25 buffaloes without any visible hydatid cysts. CIE was 69% sensitive and 84% specific.

By ELISA, 65 of the 75 sera samples from hydatid positive animals were positive, whereas only 1 serum sample from hydatid negative animals was detected positive. ELISA was 87% sensitive and 96% specific.

The protein content of the dialysed hydatid fluid from buffalo hydatid cyst was 9 mg/ml of hydatid fluid. Gatne *et al.* (1990) reported that the protein content ranged from 1.6 mg to 12 mg/ml of hydatid fluid. The protein content

estimated in the present study correlates with the earlier findings.

SUMMARY

Using counter immuno electrophoresis (CIE) and enzyme linked immunosorbent assay (ELISA), 100 sera samples collected from 75 buffaloes with known hydatid cysts and 25 buffaloes without any visible hydatid cysts on slaughter were screened. By CIE, 52 sera samples were positive out of 75 buffaloes with hydatid cysts whereas 4 sera samples were detected positive from the 25 buffaloes without visible hydatid cysts. In ELISA, 65 sera samples of the 75 buffaloes with hydatid cysts proved positive and only 1 serum sample was positive from the remaining 25 sera samples screened.

The sensitivity and specificity of CIE was 69% and 84% whereas in ELISA, it was 87% sensitive and 96% specific. Hence, the use of these serological tests may be of use in antemortem diagnosis of hydatidosis instead of knowing the disease only after the death of animals and confirmation on postmortem. Between the 2 serological tests used in the present study, ELISA is preferred to CIE owing to its better sensitivity and specificity and the only disadvantage with ELISA is the cost involvement. It is preferred in screening large number of sera samples rather than a few sera samples.

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