



International Journal of Pharma and Bio Sciences

ISSN  
0975-6299

## APPLICATION OF COUNTER IMMUNOELECTROPHORESIS (CIE) IN THE DIAGNOSIS OF HYDATIDOSIS IN BUFFALOES

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### ABSTRACT

A study was undertaken to assess the sensitivity and specificity of Counter Immunoelectrophoresis (CIE) in the diagnosis of hydatidosis in buffaloes using sera samples collected at the time of slaughter. One hundred sera samples from buffaloes were screened for hydatidosis using fertile buffalo hydatid cyst fluid as antigen by CIE. Out of the 100 sera samples used, 61 were positive for hydatid cysts in various organs confirmed on slaughter and the remaining 39 sera were collected randomly from buffaloes without any visible hydatid cysts. Forty six samples were found positive out of the 61 samples screened by CIE with 2 samples found positive out of the 39 samples from buffaloes without any visible hydatid cysts. The sensitivity and specificity of CIE in detecting hydatidosis in buffaloes was found to be 75.41 per cent and 94.8 per cent respectively.

**KEY WORDS:** Hydatidosis, Buffaloes, Immunodiagnosis, Counter Immunoelectrophoresis



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## INTRODUCTION

Hydatidosis is a disease caused by the larval stage or metacestode stage of the dog tapeworm *Echinococcus granulosus* in domestic animals particularly herbivores such as cattle, buffaloes, sheep and goats. These animals get infected by accidental ingestion of the eggs of this tapeworm from contaminated environment. The disease is of zoonotic and economic concern in food animals, since the affected organs are condemned at the time of slaughter owing to the presence of hydatid cysts particularly in lungs and liver. Most of the highly endemic areas for hydatidosis are situated in developing countries with relatively low literacy rates. The disease goes undiagnosed due to the asymptomatic nature in the affected animals and most of the time it is diagnosed only on post mortem or at the time of slaughter. Facilities to diagnose the condition ante mortem are not available and it is necessary to have a method to confirm the disease ante mortem. Immunodiagnosis is one of the reliable methods to confirm the disease and hence in the present work, Counter Immunoelectrophoresis (CIE) has been undertaken to evaluate its sensitivity and specificity in diagnosing hydatidosis using buffalo serum samples.

## MATERIALS AND METHODS

Blood samples were collected from buffaloes at the time of slaughter at the rate of 10ml blood per animal in 30ml test tubes and allowed to clot. The carcasses and the viscera of the buffaloes were inspected for the presence of hydatid cysts with particular reference to lungs, liver, spleen etc. The blood samples were refrigerated overnight and the sera separated, centrifuged at 2000rpm for 15minutes. The sera was pipetted into sterile 5ml plastic vials, preserved with Merthiolate solution at a concentration of 1: 10,000 and stored at -20°C until further use. Hydatid cysts were collected from buffaloes from the slaughter house at the time of slaughter and the 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 to a glass container and allowed to stand for few hours. The hydatid fluid was examined under the microscope to identify the presence of scolices to confirm the fertility status of the cysts. The supernatant fluid from fertile cyst was carefully aspirated and dialyzed against polyethylene glycol 6000 (Carbowax) for one hour to concentrate the fluid to half of its original volume. The concentrated hydatid fluid antigen was stored in 5ml aliquots with merthiolate (1:10,000 final concentration) as preservative at -20°C for use as antigen in CIE. Hyper immune sera raised in rabbits was used as a positive control and foetal calf serum was used as the negative control in the test. Protein content of the dialyzed hydatid fluid was estimated<sup>1</sup>.

A total of 100 sera samples were screened using CIE, of which 61 sera samples were from buffaloes with hydatid cysts confirmed on slaughter and the rest 39 samples were from buffaloes without any visible hydatid cysts. Counter Immunoelectrophoresis (CIE) was carried out as per the method described by <sup>2</sup> with few modifications using barbitone buffer as pH 8.6. One per cent agarose gel was prepared by dissolving 1000mg of agarose in 100ml barbitone buffer (pH 8.6). After heating in a water bath till the agarose dissolved completely, 3 to 4ml of molten agarose was pipetted on to microscopic slides and allowed to solidify at room temperature. Four pairs of wells of 4mm diameter each were punched in two rows with a distance of 4mm between the two wells in each pair. The wells in the cathode side were charged with the known hydatid fluid antigen. The wells in the anode side were charged with the known positive serum raised in rabbits and the suspect/test sera from buffaloes in microscopic slides. The channel of the electrophoretic tank was filled with 0.02 M barbitone buffer (pH 8.6). The slides were placed over the tank and connected by means of Whatmann No.1 filter paper strips soaked in

buffer. A current of 150volts was passed for about 45 – 60minutes. The slides were then removed and observed for precipitation line formed as a result of antigen antibody reaction. The slides were then subjected to hypertonic saline treatment to remove non specific proteins and were kept in an incubator covered with moistened filter paper for drying. The slides were stained using Amido Schwartz 10 B staining solution for 10 minutes and destained without the dye to a faint background.

## RESULTS AND DISCUSSION

Out of 100 sera samples screened from buffaloes, 61 buffaloes had cysts in various organs, and the remaining samples were chosen from buffaloes without any visible hydatid cysts. Out of the 61 known hydatid samples, 46 were detected positive by CIE where as two samples were detected false positive out of 39 hydatid negative samples giving a sensitivity of 75.41 per cent and specificity of 94.8 per cent. The protein content of the dialyzed fertile hydatid fluid from buffaloes was found to be 9mg per ml of the fluid. The protein content estimated in the present study correlates with the findings<sup>3</sup> who had reported the protein content range from 1.6mg to 12mg per ml of hydatid fluid. Many immunological tests are available for the diagnosis of hydatidosis. CIE is one such efficient, rapid, easily performable immunodiagnostic procedure which has been extensively employed in diagnosing hydatidosis particularly in human beings<sup>4, 5</sup>. CIE has also been found suitable in diagnosing hydatidosis in animals. The efficiency of CIE in diagnosing sheep hydatidosis with 92 per cent sensitivity

and 100 per cent specificity when compared with AGPT, IHA, IFAT and BFT<sup>6</sup>. In the present study, the specificity of CIE in diagnosing buffalo hydatidosis was found to be 94.8 per cent with a sensitivity of 75.41 per cent. The lower sensitivity as observed by CIE in the present study could be due to low levels of antibody titre in the infected animals<sup>7</sup>. Significantly lower antibody titres were found in sera of naturally infected animals than in experimentally infected animals. In the present study, sera from false negative buffaloes may have had a low antibody titre because of natural infection that may not have been at sufficient levels to be detected by precipitation tests such as CIE.

The false positivity as observed in the present study could be due to extensive cross reactivity between hydatid cyst fluid and other metacestode antigens<sup>8</sup>. Antigens of *Fasciola hepatica* have also been found to be cross reactive with hydatid antigens<sup>9, 10</sup>. The cross reactivity between fluid antigens of various cestodes, have been demonstrated<sup>11</sup>. These findings clearly suggest that false positivity could be common when crude antigens are used in Immunodiagnosis of larval cestode infections of food animals such as buffaloes. However, use of immunological test like CIE may be of use in ante mortem diagnosis of hydatidosis which will otherwise be confirmed only by slaughter or on post mortem. Diagnosis based on CIE may be helpful in advocating treatment to the affected animals as well in controlling the life cycle of the parasite to dogs from the infected intermediate hosts with hydatid cysts such buffaloes, cattle, sheep and goats.

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