INCIDENCE OF HYDATID DISEASE IN BUFFALOES

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ABSTRACT

The present study on incidence of hydatid disease in buffaloes was conducted to evaluate the incidence of hydatid cyst grossly as well as by means of polymerase chain reaction in buffaloes. Results indicated an overall incidence of 12% of hydatid disease in buffaloes in Akola district of Maharashtra. Liver (75%) showed the most predominant site as compared to lung. All most all the cysts were of small size (87.50%) having below 5 cm in diameter and are single in organ. Most of the hydatid cysts in liver (62.50%) did not revealed protoscolices hence considered as sterile. Each PCR isolate obtained from individual hydatdi cyst were further showed 434 base pair fragment of the mitochondrial cytochrome oxidase -1 gene on 1.5% agarose gel electrophoresis and confirmed the hydatid disease.

Keywords: buffaloes, *Bubalus bubalis*, hydatidosis, incidence, PCR

INTRODUCTION

Hydatidosis or Echinococcosis is caused by *Echinococcus granulosus* a cestode inhibiting small intestine of dog which acts a definitive host, whereas hydatid cyst is the larval stage of the canine tapeworm found in the internal organs of infected herbivores including buffaloes. Hydatidosis is found to be distributed in the worldwide especially in areas having extensive livestock production which provides suitable conditions for transmission of disease between dogs and livestock. Hydatidosis causes high financial losses in ruminants due to reduced yield and quality of meat, milk, wool, delayed performance, and condemnation of visceral organs especially liver and lungs (Benner *et al*., 2010). Singh *et al.* (2014) recorded economic losses due to cystic echinococcosis in India and suggested urgent action to control the disease. The analysis revealed total annual median loss of Rs. 11.47 billion. Cattle and buffalo industry accounted for most of the losses 93.05% and 88.88% of the animal and total losses, respectively. Present study was conducted to evaluate the incidence, organ wise involvement and fertility status of hydatidosis in buffaloes of Akola district of Maharashtra (India).

MATERIALS AND METHODS

For the present study the buffaloes slaughtered in Akola district as well the dead carcasses of buffaloes reported to the Department

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of Veterinary Pathology were observed for detailed necropsy examination. During slaughterhouse and postmortem examination, the cysts were observed for their distribution and number in different visceral organs and were carefully separated from the organ. The intact cysts recovered from infected animals were procured to the laboratory in an insulated box. Each cyst was disinfected by 70% alcohol and examined for cyst size and fertility. The fluid was collected, centrifuged and sediment was collected for further DNA isolation.

The total genomic DNA from parasitic material was isolated from protoscolices in fertile cyst or germinal layer in sterile cysts using HipurA TM Mammalian Genomic DNA Purification Kit (Himedia Ltd.) by following manufactures instructions. A region of 434 bp of the mitochondrial cytochrome oxidase -1 [COX 1] gene was amplified from each isolate using previously published primer pairs: RT_1_E.g.Cox1_F 5`-GCCATCCTGAGGTTTATGTGTT-3' and RT_1_E.g.Cox1_R 5'-CGACATAACATAATGAA AATGAGC-3' (Barnes *et al*., 2007). The PCR reaction was carried out in a final concentration of 50 µL reaction mixture containing 25 µL of Dreamtaq green pcr master mix 2× (Thermo Scientific), 18 µL of nuclease free water, 1 µL (12.5 ppm) of each primer, 5 µL of template DNA. The PCR amplification was performed as step 1 – one initial thermal cycle of 94°C for 2 minutes, 53°C for 1 minute, 72°C for 2 minutes. followed by step 2 with 35 repeated thermal cycles of 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 30 seconds. Step 3 final elongation at 72°C for 7 minutes and short-term storage at 4°C in a Mastercycler pro S and Control Panel (Eppendorf). After completion of PCR, amplified products were confirmed and analyzed by 1.5% Agarose gel electrophoresis.

RESULTS

The incidence of hydatidosis in buffaloes was estimated on the basis of necropsy examination carried out in slaughterhouses of Akola district and in the Department of Veterinary Pathology for dead carcasses. Total 96 buffaloes including 73 at slaughterhouses and 23 postmortem cases were observed for presence of hydatid cyst. Out of 96 buffaloes, 08 buffaloes were found to be infected with hydatid cyst giving an overall incidence of 12%. Among 08 hydatid cysts observed, 06 (75%) were observed in liver, one in lung (12.5%) and one in lung and liver (12.5%). Out of 08 hydatid infected buffaloes, 06 buffaloes showed single hydatid cyst however in two cases there were multiple hydatid cysts in the liver (Figure 1). Most of the hydatid cyst i.e., 07 (87.50%) were of small size (below 5 cm in diameter) while only one cyst was of medium size (5 to 10 cm in diameter). Out of 08 hydatid cyst tested for fertility, one cyst in lung (12.5%) and one cyst in liver (12.5%) showed protoscolices in microscopic examination and hence considered as fertile while 06 including one lung (12.5%) and 05 liver (62.50%) did not revealed protoscolices hence considered as sterile.

The hydatid cysts observed in 08 buffaloes were also confirmed by polymerase chain reaction (PCR). For molecular confirmative diagnosis, DNA from each cysts was extracted and a region of 434 bp of the mitochondrial cytochrome oxidase-1 [COX 1] gene was amplified from each isolate using previously published primer pairs: RT_1_E.g.Cox1_F 5`-G CCATCCTGAGGTTTATGTGTT-3' and RT_1_E.g.Cox1_R 5'-CGACATAACATAATGAA AATGAGC-3'. After completion of PCR reactions, the product size and quality were confirmed by 1.5% agarose gel electrophoresis. A 434 base pair

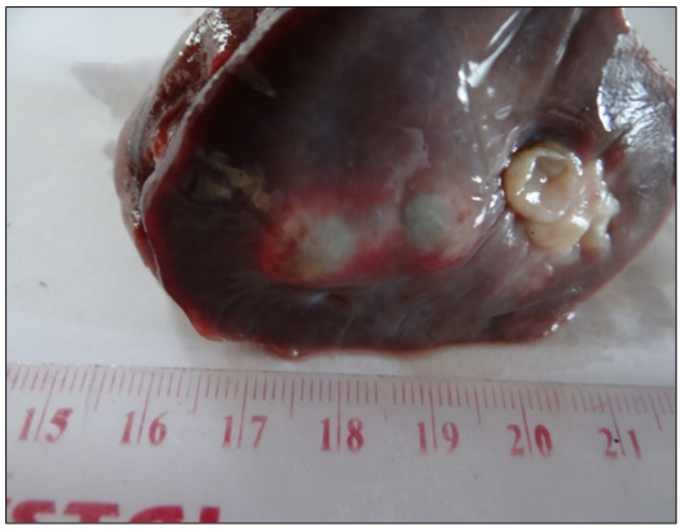

Figure 1. Liver of slaughtered buffalo showing multiple hydatid cyst.

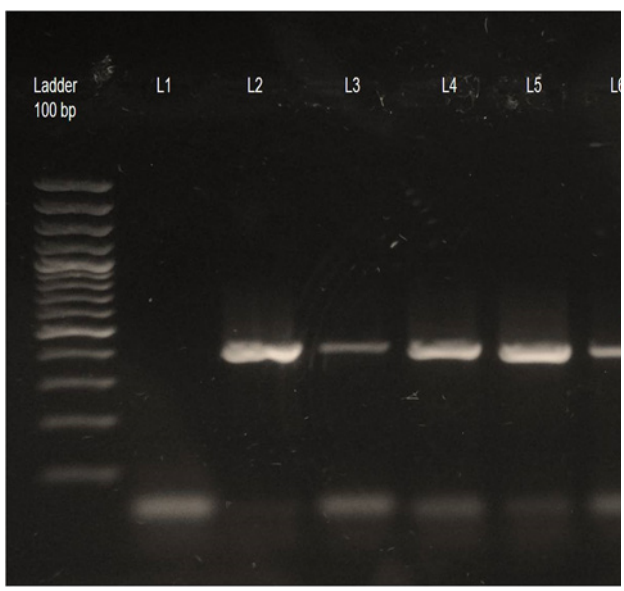

Figure 2. Gel electrophoresis (1.5% agarose gel) of polymerase chain reaction products (DNA) showing 434 bp of positive hydatid cyst samples.
L1: Negative control; L2- L6 Hydatid cyst isolates

fragment of the mitochondrial cytochrome oxidase -1 gene was obtained from each isolate and hence confirmed it as hydatid cyst (Figure 2).

DISCUSSION

Hydatidosis is one of the most important zoonotic parasitic diseases of animals and man caused by larval stage of the dog tapeworm *Echinococcus granulosus*. Intermediate host mainly sheep, goat, cattle, and buffaloes where the development of hydatid cyst mainly occurs in lungs and liver. In present study the overall incidence was recorded as 12%. Recently, Sheeba *et al.* (2016) also recorded the similar incidence of 11.11% in buffaloes at Chennai. The present findings of overall incidence of 12% of hydatidosis was comparable to the incidence reported by earlier workers Dhote *et al.* (1992), Kurkure *et al.* (1992), Pathak *et al.* (2004) in Akola region in cattle. However, literature revealed variation in prevalence of hydatidsosis rate at different locations in buffaloes could be due to variation in temperature, managemental practices, dog populations and strains of parasite.

Like the present findings Kurkure *et al.* (1992) observed liver as a most commonly affected organ (69.69%) in cattle in Maharashtra while Pathak *et al.* (2004) recorded highest prevalence in lungs (71.11%) followed by liver (62.22%), Spleen (11.11%), Kidney (6.66%) and heart (2.22%) in Akola region. Contrary to the present findings Sheeba *et al.* (2016) recorded higher incidence in lung compared to liver. The lungs and liver possess the first great capillaries sites for migrating *Echinoccoccus* onchosphere which may adopt the portal vein route and primarily negotiate hepatic and pulmonary filtering system before any other peripheral organ is involved and might be the reason for predominant location (Getaw *et al.*, 2010). Most of the hydatid cysts recorded were of small size. Similarly, Kebede *et al.* (2009), Melaku *et al.* (2012) recorded relatively smaller size hydatid cysts in liver.

In the present study, most of the cyst in liver (62.50%) appears to be sterile. Literature revealed more fertile cysts in lung compared to liver irrespective of species. The variation in rate of fertile and sterile hydatid cyst may be due to the strain differences of organism (McManus, 2006). Lung has relatively softer constancy and large capillary bed which allows easier development of cysts and fertility of hydatid cyst (Melaku *et al.*, 2012) which might be the reason for comparatively large size and fertile hydatid cyst observed in lung.

In recent years PCR is a new way of diagnosing the cestode having 100% specificity when evaluated with isolates of cestodes (Dinkel, *et. al.,* 2004). In present study each isolate amplified a region of 434 bp of the mitochondrial cytochrome oxidase-1 [COX 1] gene using previously published primer pairs. Similarly, the hydatidosis in animals was also confirmed by using the mitochondrial cytochrome oxidase-1 [COX 1] gene for PCR amplification of *E. granulosus* by Barnes *et al.* (2007), Vural *et al*. (2008), Pednekar *et al.* (2009), Moro *et al.* (2009), Sanchez *et al.* (2010) and Sharma *et al*., 2013). Bhattacharya *et al.* (2007) studied molecular analysis for 12 isolates of *E. granulosus* collected from domestic animals and analyses for DNA nucleotide sequence variation within NADH dehydrogenase subunit I (nadI), mitochondrial cytochrome c oxidase O (Cox I) and internal transcribed spacer gene I (ITS1). Hence as per literature reviewed Cox I gene can be used for molecular identification of hydatid cyst in animals by using either protocolizes or germinal wall with

high specificity and sensitivity.

In conclusion, hydatidosis is prevalent in buffaloes of Akola district and liver is the most prevalent organ with small size sterile hydatid cyst. PCR can be used for confirmative diagnosis of hydatidosis but having limitation as it requires either cyst fluid or cyst wall.

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