

Short Communication

GROSS AND HISTOPATHOLOGICAL ALTERATIONS ASSOCIATED WITH CYSTIC ECHINOCOCCOSIS IN CATTLE

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ABSTRACT: Cystic Echinococcosis also known as 'Hydatidosis' is significant helminthic zoonoses that continue to be an important problem in terms of animal as well as public health. A twelve years old, Holstein Friesian crossbred cow was presented to the Veterinary Polyclinic, Lalhri, District Una, Himachal Pradesh for post-mortem examination. External examination revealed pale-yellow mucous membranes with cachexia. Internal examination revealed the presence of variable numbers and sizes of monolocular and multilocular hydatid cysts affecting the vital organs *viz.* lungs, liver, and spleen. On cut sections, the cysts were also found deeply embedded within the parenchyma and containing protoscolices inside the fluid-filled cavity. Histopathologically, the affected organs revealed moderate to severe congestion, mononuclear cell infiltration, extensive fibrosis, degeneration, and atrophic changes in the parenchyma adjacent to the cyst. The present investigation reports an event of cystic echinococcosis affecting multiple organ systems leading to the death of cattle in Himachal Pradesh, India.

Keywords: Cattle, Cystic Echinococcosis, Gross pathology, Histopathology, Hydatidosis, Masson's Trichrome stain.

Cystic echinococcosis (CE) or hydatidosis or hydatid disease is an important and neglected zoonotic disease of helminths that threatens animal and human health worldwide including in India. It is linked with major economic losses in the livestock sector and causes a most important disease in humans [1]. In humans, the Foodborne disease burden epidemiology reference group of the World Health Organization (WHO) estimates that each year CE costs 184,000 disability-adjusted life years throughout the world [2]. Further, it is estimated that the range from 50 to 100 per 100,000 person-years in endemic foci, with 5 to 10% frequencies in certain areas of Argentina, Peru, eastern Africa, Central Asia, and China [3, 4]. In production animals, worldwide annual losses are estimated at 2 billion dollars mainly due to the losses in carcass weight, fecundity, milk production, and wool or hide production [5].

CE is caused by larval stages *i.e.* metacestodes of the tapeworm belonging to the genus *Echinococcus* of the Family *Taeniidae*. A total of 9 species of *Echinococcus* have been described so far having zoonotic implications and posing a severe threat to human health. The most important among them is *E. granulosus* which is mainly responsible for CE in animals. CE presents a serious public health threat [6], particularly in rural areas where dogs are found in close association with human beings and other domestic animals, feeding on scraps and intestines of herbivorous animals [7].

CE has carnivorous animals such as dogs as definitive hosts; and herbivores and omnivores as intermediate hosts. Usually, human beings get infected accidentally and do not play any role or part in the life cycle of this parasite. The adult stage of tapeworm, *Echinococcus*, continues living in the small intestine of

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carnivorous animals and the intermediate stage *i.e.* hydatid cysts established in the internal organs mostly liver and lungs of humans and herbivorous animals such as sheep, horses, cattle, pigs, goats, camels, etc. as fluid-filled bladders which are largely unilocular in nature [8].

In hosts, like cattle and buffalo, the disease mostly remains asymptomatic and is most commonly diagnosed at necropsy and during meat inspection in an abattoir, using visual inspection, palpation, and/or incision [9]. In addition, CE can be diagnosed in different ways using radiographs, computed tomography scans, immunoassays, serologic tests, and molecular techniques *i.e.* Polymerase Chain Reaction. Moreover, histopathological examination of the laminated cyst lined internally by a layer of epithelial cells in the sections of various organs or tissues may corroborate the identification of hydatidosis in cattle [10]. Looking at the zoonotic potential of the parasite, the present investigation was undertaken to study the gross and histopathological alterations associated with severe infection of CE in cattle.

The study

A twelve-year-old, Holstein Friesian crossbred cow was presented for the disease investigation through a routine necropsy examination at Veterinary Polyclinic, Lalhri, Distt. Una, Himachal Pradesh. A systematic and detailed post-mortem examination was conducted and the gross pathological findings in the internal organs were noticed and documented. The tissue pieces from affected organs *i.e.* lungs, liver, and spleen were further analyzed for the microscopic findings through histopathological study. The representative tissue samples of affected organs showing gross lesions were suitably collected in a 10% neutral buffered formalin solution. These tissue samples were processed with routine paraffin wax embedding technique where dehydration and clearing were done by using benzene followed by paraffin impregnation, block making, and tissue sectioning at 4-5 μm thickness. The routine Hematoxylin and Eosin staining as well as special staining *i.e.* Masson's Trichrome staining was performed as per the standard protocol given by Luna [11].

Results and discussion

According to the history of the owner, the animal was dull, depressed, had progressive emaciation, and showed signs of labored breathing before the death. On necropsy examination, subcutaneous tissues and all the visceral organs were found to be pale yellow with

remarkable hydroperitoneum and hydropericardium. The internal organs like lungs, liver, and spleen showed variable-sized, round to circular, multifocal areas of unilocular and multilocular cysts (Fig. 1a-1c). The multilocular type of cysts were the most consistent and predominant in these organs. These cysts were raised structures and bulging out from the organ surface however considerable numbers were also seen embedded deeply in the parenchyma. Cysts were filled with clear colorless to pale-yellow watery fluid and also contained a good number of protoscolices. The cut surface of these cysts was lined by a smooth membrane which could be easily peeled off (Fig. 1a-1c). Lungs and liver revealed numerous small to large sized round to circular cysts located on the surface as well as deeply embedded within the parenchyma (Fig. 1a, 1b). These cysts ranged from 2-4 cm in diameter and filled with 5 to 20 ml clear watery fluid. In a few cysts, multiple protoscolices were seen inside the fluid-filled cavity and they were attached to the internal surface. The cystic wall appeared light yellow in color. The spleen showed areas of enlargement due to the presence of multiple cysts deeply embedded within the parenchyma (Fig. 1c). The lungs of the cow were found wrinkled along with raised structures containing cysts of 3 to 5 cm in diameter and filled with 30 to 50 ml of clear watery fluid.

Microscopic examination of cysts revealed variably thickened coats of granulation tissue, causing extensive fibrosis and inflammatory cell reactions mainly composed of mononuclear cells and fibroblasts. Tissue sections of the lungs, liver, and spleen revealed variably large cyst walls with the presence of inner germinal and outer laminated layers (Fig. 2a-2d). A well-defined germinal layer was also observed separating the laminated layer in places and protoscolices in certain sections of lungs. A marked zone of inflammatory cell reaction was noted around the cystic wall which was mainly composed of lymphocytes and macrophages. The adjoining parenchyma of cysts showed pressure atrophy of alveoli by giving an appearance of slit-like structures with a narrow lumen in lung tissue (Fig. 2a). The hepatocellular degeneration, necrosis, and atrophy of surrounding parenchyma were prominent findings recorded in the liver sections. In addition, hyperplastic changes along with areas of dystrophic calcification were also seen in the cystic wall (Fig. 2c). In the present investigation, the cyst wall of the liver revealed a channel or space between the ectocyst and pericyst which was in accordance with the earlier findings in small ruminants [12]. The spleen of the animal showed

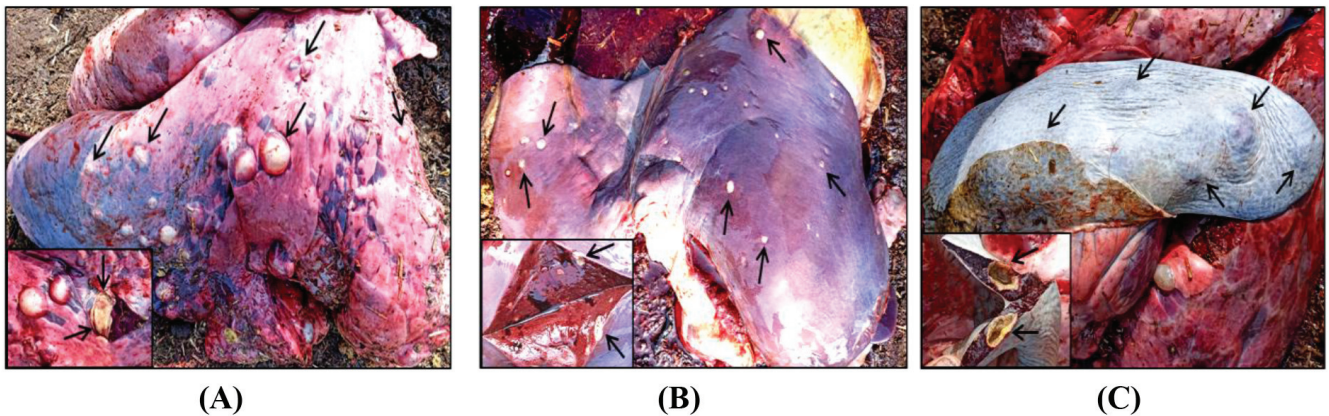


Fig. 1. Gross pathology of cystic echinococcosis in cattle. [A: Congested lungs with areas of consolidation and presence of variable-sized, round to circular, well-demarcated, raised areas of hydatid cysts (arrows) with random distribution. Inset: Cut surface revealed deeply embedded cyst within the parenchyma (arrows). B: Reduced size of liver with rough surface showing variable-sized, round to circular, well-demarcated, slightly raised areas of hydatid cysts (arrows) randomly distributed throughout the liver. Inset: Cut surface revealed well demarcated cysts embedded within the parenchyma (arrows). C: Reduced size of spleen showing raised areas of hydatid cysts (arrows) replacing the parenchyma. Inset: Cut surface revealed a well-demarcated cyst embedded within the parenchyma (arrows)].

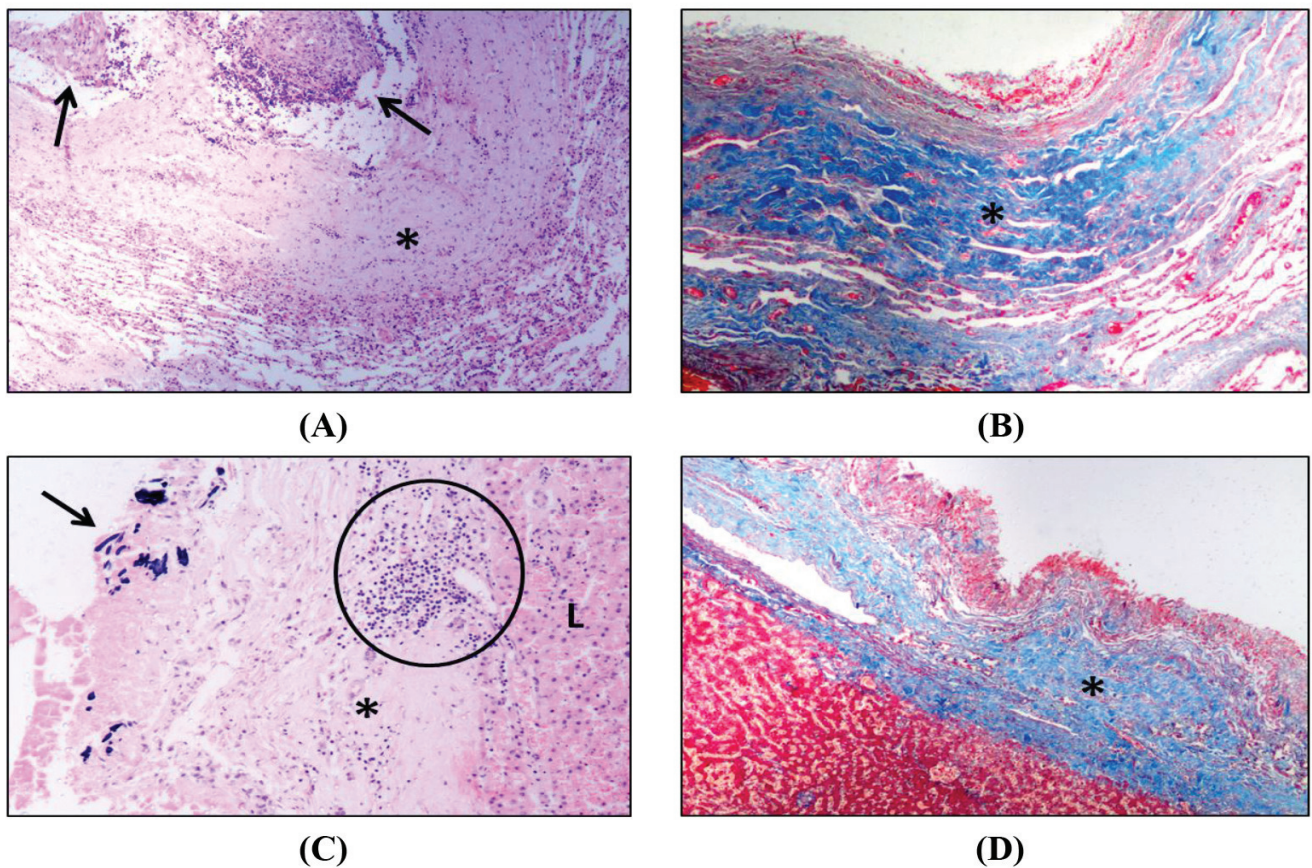


Fig. 2. Microscopic pathology of cystic echinococcosis in cattle. [A: Hydatid cyst containing fibrous connective tissue wall (*) causing pressure atrophy to the surrounding lung parenchyma. Note the cellular content admixed with inflammatory infiltration inside the lumen of cyst. H&E, 40x. B: Hydatid cyst containing fibrous connective tissue wall (*) leads to pressure atrophy of the surrounding lung parenchyma. Masson's Trichrome stain, 40x. C: Engorged and dilated vasculature and sinusoids of liver parenchyma (L) adjacent to the wall of hydatid cyst (*) along with mononuclear cell infiltration (encircle). Note the necrotic debris and calcification inside the lumen of cyst (arrow). H&E, 100x. D: Fibrous connective tissue wall of hydatid cyst (*) leads to pressure atrophy of the surrounding hepatic parenchyma. Masson's Trichrome stain, 40x].

raised areas due to the presence of multiple poorly demarcated large-sized cysts of around 5-6 cm in diameter. These cysts might have been either formed by the fusion of the multiple small-size cysts or the architectural uniqueness of this capsulated organ. The cyst wall consisted of a thick membrane due to extensive fibrosis and replaced around 20-30% of the splenic parenchyma further affecting its functional ability. Microscopically, the presence of multiple protoscolices along with laminated layers and extensive fibrous tissue proliferation encompassing the cysts were noticed. These microscopic changes during cystic echinococcosis and affecting various organs were in concordance with the previous studies in cattle [13, 14].

The liver and lungs were the most commonly affected vital organs in ruminants and the development of variable numbers and sizes of hydatid cysts in particular organs varies due to their consistency *i.e.* compactness or sponginess [12]. The extensive nature of hydatid cysts affected these organs which probably incurs noteworthy economic losses to the meat industry through condemnation of edible organs making them unfit for human consumption [15, 16, 17]. Singh *et al* [18] reported a rare case of hydatidosis affecting the spleen in a buffalo. They observed a solitary cyst protruding out from the surface of the spleen and a clear watery fluid along with protoscolices oozing out while cutting it. The inner surface of the cyst contained a white, thin, translucent membrane. Microscopically, a germinal layer along with many protoscolices was also seen. In addition, the fibrous connective tissue proliferation around the laminated layer as well as the depletion of lymphoid elements in the surrounding white follicles was also observed. In cattle, the fertile cysts revealed mixed patterns of response comprising fibrotic resolution and granulomatous response in direct contact with the laminated layer of the cyst [17]. Singh *et al* [18] found numerous cysts affecting the lungs and liver of food-producing animals. These cysts were encircled by the outer fibrous connective tissue layer above the inner germinal layer and filled with clear watery fluid. Echinococcal protoscolices were also seen inside the cysts. Additionally, in the liver, we have also noticed congestion, areas of hemorrhages, leukocytic infiltration mainly lymphocytes, and mild to moderate degeneration of hepatocytes.

The lung and liver parenchyma adjacent to cysts revealed congestion, hemorrhage, pressure atrophy, areas of mononuclear cell infiltration, and extensive proliferation of fibrous connective tissue which was

strongly evident through Masson's trichrome staining technique (Fig. 2b, 2d). Ali Ibrahim and Gameel [19] have seen variable sizes and shapes of cysts, present on the surface as well as deeply embedded in the parenchyma of the lungs or liver. These cysts were filled with clear or turbid fluid and filled with caseous material or calcified. Recently, similar gross and histopathological findings of hydatidosis were observed in liver and lung samples of slaughtered cattle collected from abattoirs in the USA [10]. However, all the cases of cysts were found to be non-fertile in nature which was in contrast to the present findings and further suggested its public health significance as well as financial consequences of this zoonotic disease.

Conclusion

In conclusion, the necropsy and histopathological findings in the present investigation revealed a severe infection of cystic echinococcosis in cattle, and the lungs, liver, and spleen were found to be the severely affected organs in Holstein Friesian crossbred cattle.

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