Indian Journal of Animal Sciences 78 (4): 373–375, April 2008

Secondary hydatidosis in Swiss Albino mice following infection with protoscolices of *Echinococcus granulosus* of buffalo origin

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Received: 1 May 2007; Accepted: 20 September 2007

Key words: Albino mice, *Echinococcus granulosus*, Pathology, Secondary hydatidosis

Hydatidosis is considered as one of the most important zoonotic diseases of global dimension and constitutes major economic problem due to presence of the larval cestode in food animals (Matossian *et al.* 1977). *Echinococcus granulosus*, a tiny cestode of canids causes unilocular type of hydatid disease in intermediate hosts, viz. sheep, goat, cattle, buffalo, pig, camel, man etc., where the hydatid cyst develops in the vital organs like liver, lungs, heart, kidney, spleen and brain. Several studies on pathological changes of spontaneous hydatidosis in farm animals have been reported (Prasad and Prasad 1980, Singh *et al.* 1988, Singh *et al.* 1992). However, experimental infection on the development of secondary cysts and changes in the tissue in laboratory animals may elucidate various aspects of the zoonotic parasites (Singh *et al.* 1988). Moreover, it is well established that *E. granulosus* exists in a complex of intraspecies variants or strains in many parts of the world (Thompson and Lymbery 1988, McManas 1996). As buffaloes are the most predominant livestock species in northern part of the Indian subcontinent, the present study deals with the development of secondary hydatidosis in albino mice with the protoscolices of buffalo origin

Isolation of protoscolices: Hydatid cysts were collected from infected liver, lungs of buffalo from local abattoir. The cyst contents were taken out aseptically with a syringe and the cysts were examined for fertility. The brood capsule containing protoscolices were isolated by sedimentation of the fertile cyst contents and washing of inner membrane of germinal layer. The protoscolices were washed 3–4 times in 0.15 M NaCl. The viability of the protoscolices was examined as per Smyth and Barrett (1980) before experimental infection.

Experimental infection: Swiss albino mice (60) were each injected intraperitoneally (i/p) with 200 viable protoscolices of buffalo origin in 0.1 ml of 0.15 M NaCl and (20) mice were kept as healthy control group each injected with only 0.1 ml of 0.15 M NaCl.

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Pathological studies: Mice infected with protoscolices were sacrificed at regular interval. First the organs viz. liver, lungs, kidney, spleen and heart, were observed for any gross pathological changes. If there were any gross changes, the part of the organs were cut and fixed in 10% neutral buffered formalin (pH–7.0). After proper fixation, washing, dehydration and clearing were carried out. Separate blocks of tissues were prepared after proper embedding. The sections were cut on a rotary microtome at 4–6 microns and were placed in a water- bath at about 50° C to flatten. The flattened sections were then placed on glass slides smeared with Mayer's egg albumin and dried. The sections were then stained with haematoxylin and eosin (H & E) for routine and histopathological studies.

Gross changes were seen in liver, lungs, spleen and kidney of infected mice from 3 months onwards. At 3 months post infection (pi), small white spots of echinococcal foci (<1 mm) were recorded on the surface of these organs. Most of the cystic foci were found in liver, followed by spleen, kidney and lungs. Maximum number of 8 cysts were recovered from an infected mouse at 6 month p.i and were the size of >1 mm. Cyst or cystic foci could be recorded in 66.66% of infected mice. Grossly, hepatomegaly and splenomegaly were the predominant changes in experimental infection and the enlargement of the spleen was 2 to 3 times in comparison to the non-infected mice. None of the cysts developed to maturity as brood capsule and protoscolices were not been detected. Histological picture of liver of infected mice revealed vascular degenerative lesions with calcified cystic materials. Mild degenerative changes in the form of cloudy swelling of hepatocytes were observed. Multiple cystic foci with calcareous deposits were not uncommon in the liver parenchyma. In portal triad areas, infiltration of mononuclear cells and engorgement of blood vessels was a frequent finding. Collapsed cysts were seen embedded in the lung substances with several layers of reticuloendothelial cells. Many bronchioles were also filled with RBCs and fibrinous exudates which showed infiltration of polymorphs and mononuclear cells. Histological picture of lungs revealed

thrombosis and accumulation of mononuclear cells. Histopathologically, the kidneys of infected mice revealed large cystic spaces in the parenchyma. Adjacent cells of tubular epithelium showed degenerative changes. Kidney cortex showed intense reaction along with mononuclear cell infiltration around the degenerated cyst. Spleen of infected mice revealed lymphoid hyperplasia and multiple foci of calcareous deposits. Destruction of erythrocytes and large number of megakaryocytic cells were also noticed .

From the result, substantial changes in both gross and histological picture of the organs of infected mice and echinococcal foci or cysts were recorded on the surface of liver, spleen, kidney and lungs of mice. This corroborates with the reports of Alkarmi and Ali-Khan (1984), Singh and Dhar (1991), Matsumoto *et al.*(1998). Secondary cyst development occurred in 66.6% of mice in the present study while Harnandez and Nieto (1994) reported that infected group exhibited 69% susceptibility of infection in outbred CDI mice which was comparable to the present findings. However, Sungur (1979) recorded as high as 92.5% secondary cyst while Madhubala *et al.* (1993) could record it in only 15.9% of infected mice. The greater percentage reported by Sungur (1979) might be due to the infection with a higher dose of protoscolices (1000 PSCs) and strain variant (sheep origin). In contrast to this, bovine and buffalo strain which were used for the experimental infection by Harnandez and Nieto (1994) and Madhubala *et al.* (1993) and also in the present studies from buffalo origin may be of less pathogenic nature resulting into less development of echinococcal foci. Further experimental studies on the development of secondary cysts and changes in the tissue in laboratory animals will elucidate various aspects of the zoonotic parasite. Moreover, it is well established that *E. granulosus* exist in a complex of intraspecies variants or strains in many parts of the world (Thompson and Lymbery 1988, Mcmanas 1996). Splenomegaly and hepatomegaly were the predominant gross changes in the infected mice. Similar findings were obtained in albino mice, guinea pigs and golden hamsters infected with 200 PSCs of sheep origin (Singh *et al.* 1988, Singh and Dhar 1991). From the present results, it appears that some cysts frequently manifested regressive changes as pseudo-retention of fluid substance or collapse of the cyst. The presence of calcareous substances in the multiple cystic foci at the later stage also gave indication of regressive changes. Swiss albino mice might not be a suitable host/model as the cyst formed did not progress to maturity were in accordance with the observations made by Singh *et al.* (1988). Susceptibilities among the 4 inbred strains of mice for a suitable host for secondary hydatidosis, BALB/C and A/J mice were found to represent extreme susceptible and resistant, respectively(Emery *et al.* 1997). Studies of Osman *et al.*(1998) indicated that the development of cyst in terms of number, size and maturity was better obtained in *Cricetulus migratorius* than NIH mice, while AKR mice was reported to be best model among the other strains of mice for *E. multilocularis* (Matsumoto *et al.* 1998). Refractory or partially insusceptible hosts restrict the development of larval cyst mass by intensifying a massive tissue reaction against the pathogen whereas in hyper susceptible host an insignificant tissue reaction may occur around the larval cyst mass. The relative resistance to parasite development might be associated with the T cell response characterized by the production of high level of Th–1 cytokines (Emery *et al.*1997). Experimental infection and development of secondary cysts may therefore be determined by various factors, viz. experimental animal (species or strain), type of infection (protoscolices/oncosphere of *E.granulosus/E.multilocularis*), the dose, route and strain of infection. Besides this, experimental design and individual biological difference can also be accounted for variation in such complex host parasite relationship.

SUMMARY

Swiss albino mice were experimentally infected with protoscolices of *Echinococcus granulosus* of buffalo origin through intraperitoneal route. From 3-month post infection (p.i), small spots of echinococcal foci (<1 mm) were recorded on the surface of liver, lungs, spleen and kidney. At six-month infection, maximum number of cysts (> 1 mm) were recovered. Cysts or cystic foci were recorded from 66.66% of infected mice. Splenomegaly and hepatomegaly were the predominant gross changes in experimental infection. Histopathological changes in the organs of mice due to secondary hydatidosis have been discussed in the paper.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Indian Veterinary Research Institute for providing all facilities to carry out the present investigation.

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