Bile Duct Hyperplasia and Associated Abnormalities in the Buffaloes Infected With *Fasciola gigantica*

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Abstract -- Liver samples of buffaloes infected with *Fasciole gigantica* were collected from the main abattoir of Hyderabad City. Histological studies of infected livers revealed severe damage resulting in disrupted hepatic cords, inflammation, atrophy and necrosis. Bile duct hyperplasia was prominent with proliferation of epithelial cells.

Key words: Fascioliasis, hyperplasia, bile ducts.

INTRODUCTION

Fasciola hepatica and Fasciola gigantica are the causative agents of the disease fascioliasis in the live stock of Pakistan (Kendall, 1954). The economic losses due to fascioliasis are quite evident (Chaudhry et al., 1984; Mehra et al., 1999; Anonymous, 2003). Hyperplasia is a condition, which is characterized by increase in the number of cells in the tissue or organ. Proliferating cells are similar to their parent cells and of regular size and shape. These cells are capable of mitotic division and co-ordinate with the function of parent tissues. Hyperplasia occurs due to increased hormonal stimulus, physical or chemical function (Robbins and Kumar, 1994). One of the common causes of hyperplasia is chronic irritation. Induced hyperplasia of epithelium is caused by parasitic infestation in various organs (Cheville, 1989).

Chronic inflammation, chronic injuries and regenerative hyperplasia of the bile duct epithelium may be related to malignant transformation (Shimonishi *et al.*, 2000). Recent reports have identified *Fasciola hepatica* as a neoplastic risk agent, primarily in animals, which could cause the genetic damage of the surrounding host tissue (Gentile *et al.*, 1998; Motorna *et al.*, 2001). Pathology in cattle and buffaloes due to fascioliasis has been described previously (Keck and Supperer, 1967; Sabri *et al.*, 1982; Swarup and Pachauri, 1987). Present investigation was undertaken to describe bile duct hyperplasia and related abnormal-

0030-9923/2004/0003-0231 \$ 4.00/0 Copyright 2004 Zoological Society of Pakistan. lities due to infection caused by *Fasciola gigantica* in buffaloes.

MATERIALS AND METHODS

Buffaloes were examined for the liver fluke infection at the main abattoir of Hyderabad City. Visual examination technique was used for random collection of the infected livers. Liver flukes from the infected livers were collected and preserved in 70% alcohol. These were then processed for staining in Carmine Alum and finally mounted permanently in Canada Balsam. The flukes were then identified with the help of keys and relevant literature (Soulsby, 1982; Yamaguti, 1985).

For histopathology, pieces from 10 infected livers were taken and immediately fixed in 10% formaline. Sections of 6-8 μ m thickness were prepared with paraffin embedded tissue by using standard histological techniques (Bilqees and Fatima, 1993). Sections were stained with haematoxylin and eosin and mounted permanently in Canada Balsam. Microphotographs of the tissue sections were prepared for histological observations.

RESULTS

A total of 330 buffaloes were examined for the liver fluke infection. The liver flukes, recovered from the buffaloes, were identified as *Fasciola gigantica*. No other species of *Fasciola* was recorded from these hosts during this investigation. The prevalence of the infection was 14.8%.

Gross pathology of the infected livers was recorded before processing for histopathological studies. Infected livers were obviously swollen showing the condition of hepatomegaly. Light and dark patches were clearly visible on the surface of the liver. In the cut section, the swollen and the fibrotic bile ducts were prominent and blocked with infolded flukes. The bile ducts were found filled with blackish brown exudate (Fig. 3). Upon incision of the liver, the flukes were visible moving in the tissue.

Histopathological observations, based on the serial sections of the infected liver tissues, revealed severe destruction in the liver tissue including inflammation, atrophy, necrosis, fibrosis and hyperplasia of the bile ducts. Infiltration of the inflammatory cells including macrophages and plasma cells was observed in all sections. The infiltration of these cells was severe in the area of portal triads.

Bile ducts manifested hyperplastic changes in the epithelium (Fig.4). Normally these ducts are lined with elongated epithelial cells having large nuclei at the base (Figs. 1 and 2) but in the infected tissues, the bile ducts were of different size and shape with the dilated lumen, which showed the increased number of epithelial cells or hyperplasia. In some sections, proliferation of these ducts with the infiltration of the inflammatory cells was observed (Fig.5). Severe fibrosis occurred in those bile ducts, which contained flukes (Fig. 6). These bile ducts also possessed dilated lumen and were found surrounded by fibrinous exudate.

Normal architecture of the hepatic cord was distorted (Fig. 7). Hepatocytes were found compressed, atrophied and dislodged from their normal position. Empty spaces in the area were due to compression and atrophy of the cells. Disintegration of the bile ducts was observed. The nuclei of the epithelial cells of these bile ducts were found grouped into masses.

DISCUSSION

Fascioliasis is a common disease of the livestock, which has been reported from all the provinces of Pakistan (Kendall, 1954; Chaudhry and Niaz, 1984). It is evident from the literature that the buffaloes are more susceptible to fascioliasis with higher incidence of this infection (Bilqees and Alam, 1988, 1991; Shaikh and Khan, 2000). Prevalence in buffaloes was reported previously as 9.40% (Sabri *et al.*, 1981); 19.5% (Masud and Majid, 1984); 8.50% (Bilqees and Alam, 1988) and 8.61% (Bilqees and Alam, 1991), which are more or less similar to the present investigation (14.8%). The variations in the prevalence of fascioliasis may be attributed to the meteorological factors and grazing habits of the host.

Histopathology of the liver in fascioliasis have been studied by many workers in varieties of the hosts including cattle and buffaloes (Dwivedi and Singh, 1965; Rahko, 1969; Armstrong and Millar, 1980; Sabri *et al.*, 1982; Haroun *et al.*, 1986; Swarup and Pachauri, 1987; Fahmy and El-Attar, 1990; Lopez *et al.*, 1993; Gunsser *et al.*, 1999; Hamir and Smith, 2002). The general pathology of the infected liver of buffaloes was more or less similar to the earlier observations (Rahko, 1969; Sabri. *et al.*, 1982; Swarup and Pachauri, 1987), however it differed in the nature of severity of the damage caused to the tissue.

Present study revealed severe destruction of hepatic tissue and bile ducts. Inflammation, necrosis, disruption of the normal hepatic cord and fibrosis were also observed. In addition, bile duct hyperplasia was prominent in all the liver sections.

Liver damage results due to many causes including hepatitis and infection (Robbins and Kumar, 1994). Pathological changes due to fascioliasis is mainly due to the infection and migration of immature liver flukes through the tissue causing haemorrhage and irritation, which bring about the inflammatory reactions (Doy and Hughes, 1984; Wiedosari et al., 1991). The development of hyperplasia of the bile duct has earlier been observed in mouse, rat, sheep and other hosts (Dawes and Hughes, 1964). The epithelium becomes folded into numerous crypt-like formation when the liver flukes entered the bile duct. This damage was caused by the spiny surface of the flukes, and their feeding habits on the hyperplastic bile ducts. Other reports have shown that the elevated concentration of praline, which is released in a large quantity by the liver fluke, induces the deposition of collagen and hyperplasia (Wolf-Spengler and Isseroff, 1983; Modavi and Isseroff, 1984).

Fig. 1. Histological micro-photograph of normal buffalo liver (Control). Magnification: x 100.

Fig. 2. Portal tract area from liver of buffalo magnified. Note, bile duct hepatic vein and artery. Magnification: x 400.

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Fig. 3. Portion of the infected buffalo liver showing dark and light patches on the surface and thickened fibrotic bile ducts.

Fig. 4. Hyperplasia of the bile ducts in the liver of buffalo . Note, some disintegrating bile ducts, necrotic area and inflammatory cells. Magnification: x 50.

Fig. 5. Proliferation of the bile ducts and infiltration of the inflammatory cells in liver of buffalo. Magnification: x100.

Fig. 6. Fibrosis of the bile duct in liver of buffalo. Section of the fluke is visible in the dilated lumen. Magnification: x10.

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Fig.7. Bile ducts disintegration and architectural distortion of hepatic cords. Note, compressed and atrophied hepatocytes. Nuclei of the disintegrating ducts seen as a mass Magnification: x100.

One of the mechanisms of pathogenecity and virulence, which is firmly established, is the harmful effect of toxic products, produced by the parasites. Adult liver fluke is a blood-sucking parasite, which produces toxic and metabolic products (hemolysin, praline, etc), which affect the liver tissues and causes necrosis and disintegration of the tissues.

It is evident from the study that *Fasciola* gigantica causes severe damage to the liver tissues including hyperplasia of the bile duct, inflammation, atrophy, fibrosis and disrupted hepatic architecture, which definitely affect the health and vital activity of the animal.

REFERENCES

- ANONYMOUS., 2003. Fascioliasis causes losses in cattle, sheep and camelids. *Vet. Rec.*, **152**: 189-192.
- ARMSTRONG, D. AND MILLAR. C. R., 1980. Liver fluke in cattle, life cycle, pathology, economic impact. *Norden News*, 55: 30-33.
- BILQEES, F. M. AND FATIMA, H., 1993. *Microtomy and histopathology techniques*. pp. 1-16. MAH Qadri Biological Research Centre, University of Karachi.

BILQEES, F. M. AND ALAM, M. A., 1988. Fascioliasis in the

live stock in Karachi. Proc. Parasit., 5: 15-36.

- BILQEES, F. M. AND ALAM, M. A., 1991. Problem of fascioliasis in cattle in Karachi. Proc. Prasit., 11: 71-77.
- CHAUDHRY, N. I., MAHMOOD, M. AND AHMED, S., 1984. A note on economic losses due to fascioliasis in cattle and sheep. *Pakistan vet. J.*, **4**: 45-46.
- CHAUDHRY, A. H. AND NIAZ, M., 1984. Liver fluke a constant threat to live stock development. *Pakistan vet. J.*, **4**: 42-43.
- CHEVILLE, N. M., 1989. *Cell pathology*, 2nd Ed. Iowa State University Press.
- DAWES, B. AND HUGHES, D. L., 1964. Fascioliasis: The invasive stages of *Fasciola hepatica* in mammalian hosts. *Adv. Parasitol.*, 2: 97-165.
- DOY, T.G. AND HUGHES, D.L., 1984. Early migration of immature *Fasciola hepatica* and associated liver pathology in cattle. *Res. Vet. Sci.*, 37: 219-222.
- DWIVEDI, J. N. AND SINGH. C. M., 1965. The occurrence of Fasciola hepatica in the lungs of Indian buffaloes (Bos bubalis). Indian vet. J., 42: 662-663.
- FAHMY, M. F. M. AND EL-ATTAR, S. R., 1990. Pathological study on fascioliasis in camel and solipeds. *Egyptian J. comp. Path. clin. Path.*, 3: 285-291.
- GENTILE, J. M., GENTILE, G. J., NANENGA, B., JOHNSON, M., BLANKESPOOR, H. AND MONTERO, R., 1998. Enhanced liver cell mutations in trematode infected Big-blue transgenic mice. *Mutat. Res.*, 400: 255-360.

- GUNSSER, I., HANICHEN, T. AND MAIERL, J., 1999. Liver fluke infestation in New World camelids. Parasitology, pathology, clinical findings and therapy. *Tierarztl. Prax. Ausg. G Grosstiere Nutztiere*, **27**: 187-192.
- HAMIR, A.N. AND SMITH, B.B., 2002. Severe biliary hyperplasia associated with liver fluke infection in an adult alpaca. *Vet. Rec.*, **39**: 592-594.
- HAROUN, E. M., HAGA, A.G. AND GAMEEL, A. A., 1986. Studies on naturally occurring ovine fascioliasis in Sudan. *J. Helminth*., **60**: 47-53.
- KECK. G. AND SUPPERER. R., 1967. Studies on the large liver fluke (*Fasciola hepatica*). Bile duct regeneration after elimination of calcium deposits. *Vet. Bull.*, **37**: 26-95.
- KENDALL, S. B., 1954. Fascioliasis in Pakistan. Ann. trop. Med. Parasit., 48: 304-313.
- LOPEZ, P., TUNON, M. J., GONZALEZ, P., DIEZ, N., BRAVO, A. M. AND GONZALEZ G.J., 1993. Ductular proliferation and hepatic secretory function in experimental fascioliasis. *Ex. Parasit.*, **77**: 36-42.
- MASUD, F. S. AND MAJID, A., 1984. Incidence of fascioliasis in buffaloes and cattle of Multan Division. *Pakistan vet. J.*, **4**: 33-34.
- MEHRA, U. R., VERMA, A. K., DASS, R. S., SHERMA, R. L. AND YADAV, S. C., 1999. Effects of *Fasciola gigantica* infection on growth and nutrient utilization of buffalo calves. *Vet. Rec.*, **145**: 699-702.
- MODAVI, S. AND ISEROFF, H., 1984. *Fasciola hepatica*. Collagen deposition and other histopathology in the rat host's bile duct caused by the parasite and proline infusion. *Exp. Parasit.*, **58**: 239-244.
- MOTORNA, O.O., MARTIN, H., GENTILE, G. J. AND GENTILE, J. M., 2001. Analysis of lac1 mutations in Big-blue transgenic mice subjected to parasite-induced inflammation. *Mutat. Res.*, **484**: 69-76.
- RAHKO, T., 1969. The pathology of natural *Fasciola hepatica* infection in cattle. *Path. Vet.*, **6**: 144-256.
- ROBBINS, S. L. AND KUMAR, V., 1994. Basic pathology,

5th Ed. W.B. Saunders, Philadelphia, PA.

- SABRI, M. A., KHAN, M. Z., CHAUDHRY, A. H. AND SAMAD, H. A., 1981. Studies on the incidence and taxanomy of *Fasciola* species in buffaloes and cattle. J. Anim. Sci. Pak., 3: 49-54.
- SABRI, M. A., KHAN, M. Z., CHAUDHRY, A. H. AND SAMAD, H. A., 1982. Comparative pathology of natural fascioliasis in buffalo and cattle. *Pakistan vet. J.*, 2: 27-28.
- SHAIKH, A. A. AND KHAN, M. M., 2000. Incidence of fascioliasis in buffaloes slaughtered at Hyderabad abattoir, Sindh Pakistan. Sindh Univ. Res. J. (Sci. Ser)., 32: 23-26.
- SHIMONISHI, T., SASAKI, M. AND NAKANUMA, Y., 2000. Precancerous lesions of intrahepatic cholangiocarcinoma. J. Hepatobil. Pancreat. Surg., 7: 542-550.
- SOULSBY, E. J. L., 1982. Helminths, arthropods and protozoa of domesticated animals. 7th Ed. Bailliere, Tindall and Cassell. Ltd. London.
- SWARUP, D. AND PACHAURI, S. P., 1987. Biochemical, histopathological and histochemical changes in liver of buffaloes affected by fascioliasis. *Indian J. Anim. Sci.*, 57: 1077-1082.
- WIEDOSARI, E., GRAYDON, R., COPEMAN, D. B., 1991. Comparative pathological study of hepatic changes induced by *Fasciola gigantica* and *Gigantocotyle explanatum* in Javanese thin tailed sheep. *J. comp. Path.*, **105**: 147-155.
- WOLF-SPENGLER, M. L. AND ISSEROFF, H., 1983. Fascioliasis : Bile duct collagen induced by proline from the worm. J. Parasit., 69 : 290-294.
- YAMAGUTI, S., 1985. Systema helminthum. The digenetic trematode of vertebrates Part I and II. Interscience Publisher, New York.

(Received 31 January 2004, revised 24 March 2004)