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Histopathological Changes in the Liver of Buffaloes by Digenetic Trematode *Paramphistomum cervi*

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Abstract: Hepatic damage in buffalo infected with *Paramphistomum cervi* is described here based on the study of histological sections of infected liver of the animal. Hundreds of parasites were found attached to the liver. Pieces of infected liver were processed for section cutting and enclosed in paraffin wax by usual process. 5-7 micron thick sections were prepared, dewaxed, stained with hematoxylin and eosin by standard method and mounted permanently in Canada balsam. Microphotographs of selected portions of the sections were prepared in support of observations. The observations revealed severe tissue damage and bile duct hyperplasia. Hardly normal hepatocytes were seen. Most of the liver tissue were replaced by proliferating bile duct, necrotic material and light to heavy fibrosis of the portal tract area. In severe infections even the bile ducts were disintegrated with separation of epithelial cells and their nuclei.

Key words: Buffaloes, liver, digenetic, trematode, *Paramphistomum cervi*

Introduction

Parasitic diseases are a global problem and considered as a major obstacle in the health and products performance of animals. Loss of millions of rupees occur due to infections in animals like buffaloes which are a great source of meat, dairy products, wool and hides throughout the world. Retarded growth, poor production of milk, meat, wool, poor quality of skin and hides are known in bovines due to parasitic infections (Sarwar, 1962; Khan, 1963; Ghori *et al.*, 1968; Ahmed, 1980; Horal, 1971). These are the end result of traumatic and toxic damages caused in liver and other organs by the parasites. Fascioliasis is already well known for such damages. But relatively little is contributed in the literature about the tissue damages caused by *Paramphistomum cervi* and almost nothing from Pakistan. Although it is a common parasite of liver and associated organs in Pakistan as in several other countries. This infection is present at least in 50% of bovines specially in buffaloes which are slaughtered at local slaughter houses as revealed by frequent slaughter house visits. Therefore, it was desirable to study the damages caused to the liver of buffalo by *P. cervi*. As liver is the main source of energy for the body of animals. Damage to liver means impaired energy supply to the body and health problems.

Materials and Methods

Severely infected liver of buffaloes were used for histological study which were acquired by local slaughter houses. Pieces of selected liver were fixed in 10% formalin for 24 h, washed several times with 70% alcohol, dehydrated through the graded series of alcohols cleared in cedarwood oil, then left in xylene for 15 min and washed twice in fresh xylene for complete removal of the cedarwood oil. Then left in a mixture of xylene and melted wax (50:50) for overnight, shifted to pure wax leaving in an incubator for 8 h. Specimen blocks were made in cavity blocks by usual procedure, trimmed in rectangular pieces and 5-7 micron thick sections were cut by a rotary microtome. Wax ribbons containing the specimens were stretched by hot water and arranged on clear slides with small quantity of egg albumin on the surface to avoid removal of the specimen sections during processing. These slides were stored for 24 h, drying at room temperature. These were dewaxed and stained with hematoxylin and eosin and mounted permanently in Canada balsam by standard method.

Photographs of selected portions of the sections were prepared in support of the observations using Nikon-Optiphot-2 photomicroscope and Fuji colour film.

Results

The histopathologic observations of the infected liver of buffalo has revealed that serious damage has occurred due to this infection. In most of the sections, the normal architecture of liver tissue, arrangement of hepatocyte cords, central veins and portal tract areas were undergone total destruction (Fig. 1, 2). A massive fibrous scar was

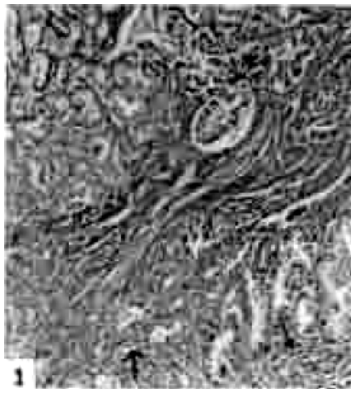

Fig. 1: Section of liver of buffalo infected with *Paramphistomum cervi*. Note the severe damage, profuse branching of bile duct, necrosis of liver tissue (arrow) and bridging fibrosis ×20

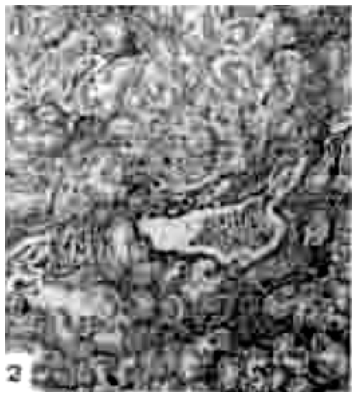

Fig. 2: Section of liver showing bile duct hyperplasia and disintegration of epithelium of ducts. Slugging of the vein is also obvious. No normal liver cells are preserved ×20

Fig. 3: Similar section as in Fig. 2 at an higher magnification showing the swollen epithelial cells and disintegrating bile ducts (arrow) ×50

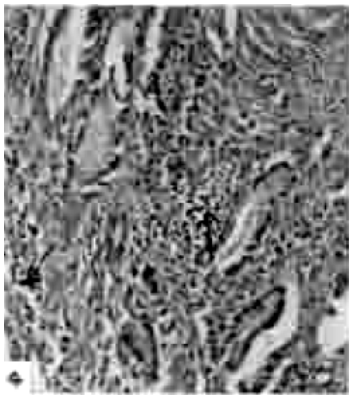

Fig. 4: A section of liver at higher magnification showing necrosis of bile duct with large number of inflammatory cells which can be seen throughout the section (arrow) ×50

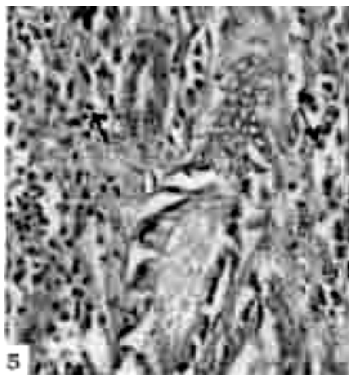

Fig. 5: Portion of Fig. 4 enlarged showing severe necrosis of the tissue and destruction of the bile ducts with separation of nuclei (arrow) ×100

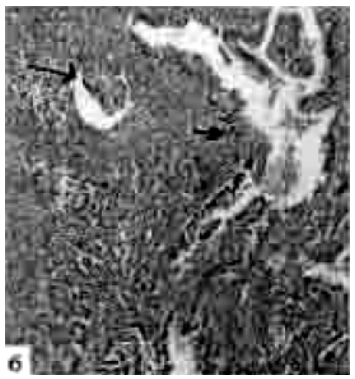

Fig. 6: Shows chronic passive congestion and necrosis of the centres of liver lobules. Degeneration of large bile duct is also obvious. Another section of bile duct shows dislocation from the surrounding tissue (long arrow). The portal tract is rimmed with a mononuclear infiltrate (small arrow) ×20

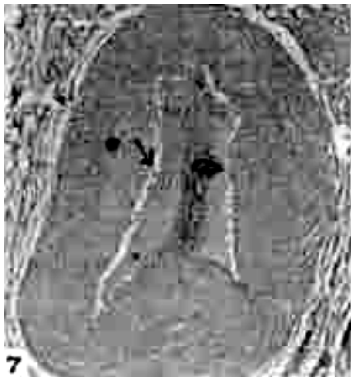

Fig. 7: Complete obliteration and formation of thrombus in an artery, narrow rim of passage is seen at the surface of thrombus (arrow). Note the surrounding massive fibrous scars ×20

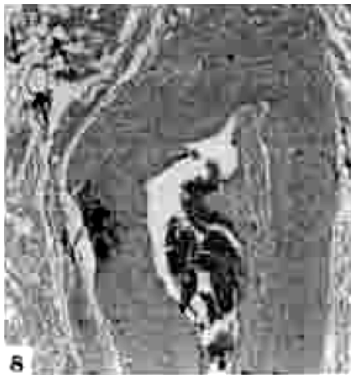

Fig. 8: Section showing thickening of walls of an artery and atherosclerotic plaque partly occluding its lumen. Note the degenerate hepatocyte in the surrounding area, many vacuoles of varying sizes and empty spaces (arrow) ×20

obvious with crowded bile ducts indicating bile duct hyperplasia, necrosis of liver tissue and bridging necrosis, a zone of intracellular necrosis existing from that central vein to the portal tract was also noted (Fig. 1). Disintegration of epithelium of bile ducts was prominent with slugging of the vein in portal tract area (Fig. 2). At higher magnification bile duct epithelium appeared swollen with dislocation of the ducts from the surrounding tissue leaving a clear space (Fig. 3). Necrosis of bile duct and necrosis of surrounding hepatic tissue was prominent in several sections with infiltration of numerous inflammatory cells (Fig. 4). There was no trace of normal hepatic tissue. The bile ducts were also destroyed leaving separate nuclei in groups. In some of nuclei chromatin was also lost. The epithelial cells were also atrophied and separated leaving clear spaces in between the tissue. Inflammatory cells mostly lymphocytes and several macrophages were prominent (Fig. 5). Several sections indicated marked chronic passive congestion and necrosis of the centres of the liver lobules. Large bile ducts were also affected. The portal tract rimmed with a mononuclear infiltrate. Hepatocytes show focal necrosis and an inflammatory infiltrate. In these sections no normal hepatocytes were preserved (Fig. 6). Blood vessels were also affected. Large vessel revealed marked fibrous thickening of its wall. Obliteration and formation of thrombus in the blood vessel leaving a narrow rim of passage, was a common finding. In these sections surrounding tissue showed massive fibrous scars (Fig. 7). Other sections showed fibrous thickening of arterial wall with atherosclerotic plaque and part occlusive thrombus with fibrous scaring in its vicinity, separating islands of degenerating hepatocytes, many of which were containing vacuoles of varying size and with several large spaces in the surrounding area (Fig. 8).

Discussion

The pathogenicity of Paramphistomiasis is directly proportional to the number of worms. The pathogenic action of the immature paramphistomes in the small intestine is mechanical and toxic. Same appears true for hepatic amphistomiasis caused by *P. cervi*. Numerous flukes were found attached to the liver of buffaloes causing inflammation, infiltration of cells and necrosis. Bile duct hyperplasia was a common finding in the tissue sections. In the literature histopathology of intestinal paramphistomiasis caused by species other than *P. cervi*, has been reported in detail by various authors in several countries (Dawes, 1936; Varma, 1957; Boray, 1959). Sahai *et al*. (1985) studied certain histochemical alterations in the different tunics of duodenum due to *P. cervi*. Singh *et al*. (1984) also reported histopathology of the duodenum and rumen of goats during experimental infections with *P. cervi*. Present observations indicate that damage caused due to *P. cervi* is more or similar to that of *F. hepatica*as far as hyperplastic and hypertrophic bile duct mucosa and cirrhosis in the affected areas is concerned (Sabri *et al*., 1982).

But there is no information available on hepatic histopathology caused by *P. cervi* in buffalo or other ruminant. During the present observations it was noted that severe destruction has occurred, in the liver of buffalo infected with *Paramphistomum cervi*. It appears due to presence of large number of trematodes attached

to the organ. The histological sections revealed that bile duct hyperplasia was predominant following destruction of bile ducts. Normal hepatic pattern was obliterated and there was no trace of normal hepatocytes in almost all of the sections. The liver tissue was replaced by necrotic material and massively congested and necrotic lobules and infiltrated masses of inflammatory cells. Bridging necrotic patterns and fibrous scars in the tissue were common findings. It is known that frequently considerable bile duct hyperplasia occurs in cirrhosis of liver. In the present study bile duct hyperplasia and destruction of parenchyma and its replacement by fibrous tissue has occurred due to which normal architecture of the liver was disrupted. Bile duct hyperplasia is also known in fascioliasis and similar pathological liver conditions have been described (Uzoukwu and Ikeme, 1978). Present observations indicate liver failure resulting from hepatocellular necrosis causing widespread destruction of parenchymatous tissue and cirrhosis.

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