PREVALENCE AND PATHOLOGY OF AMPHISTOMOSIS IN BUFFALOES AND CATTLE IN PAKISTAN

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ABSTRACT

Prevalence and pathology of biliary amphistomosis was investigated in 341 and 189 freshly slaughtered buffaloes and cattle, respectively. The prevalence was 75.07 and 50.7% in buffaloes and cattle, respectively. The prevalence was significantly higher ($P<0.0001$) in buffaloes as compared to cattle. The infected bile ducts in buffaloes were increased in length and diameter due to hyperplasia. In cattle, the walls of infected bile ducts were thickened and calcified and formation of papillary processes was evident. Hyperplasia of biliary mucosa, was observed in the buds of mucosa associated with the acetabula of the attached parasites.

INTRODUCTION

Amphistomes or conical flukes are small trematodes which are ubiquitous in large and small ruminants. Species belonging to four genera, namely *Paramphistomum, Gastrothylax, Cotylophoran* and *Gigantocotyle* have been examined in considerable detail (Soulsby, 1982). The adults of first three genera occur in the rumen while those of *Gigantocotyle* are found in the bile ducts. All four genera have been reported from buffaloes and cattle in Pakistan (Yusuf and Chaudhry, 1970).

The life cycle of amphistomes is similar to those of *Fasciola*. spp; perhaps for this reason the two infections often occur together in the same animal. Snails belonging to genera *Bulinus, Indoplenorbus* and *Lymnaea* have been incriminated as intermediate hosts. Upon ingestion by a suitable host, the metacercariae pass through the rumen, abomasum and intestine where excystment occurs and young amphistomes become attached to the intestinal mucosa and produce severe acute disease. After reaching a certain size, the flukes migrate to bile ducts and rumen and cause chronic amphistomosis.

The immature amphistomes cause sporadic epizootics of acute gastroenteritis with profuse, foetid diarrhoea and 21-37% mortality in young stock (Horak, 1971). Such epizootics have not been reported in Pakistan. The lesions of chronic biliary amphistomis, caused by adult *Giganthocotyle* spp. have been partially recorded by Eduardo and Manual (1979), Ghosh *et al.* (1982), Swarup *et al.* (1987), Hafeez and Rao (1989) and Khan and Anjum (1994). These included distention of bile ducts, hyperplasia and desquamation of mucosa and whitish foci in liver. This study presents prevalence and pathology of chronic biliary amphistomosis in buffaloes and cattle in Pakistan.

MATERIALS AND METHODS

Sihala abattoir at Islamabad was visited twice a week from December 1987 to September 1988 for the collection of samples. Rumens and livers were examined from 341 and 189 freshly slaughtered buffaloes and cattle, respectively. Location and severity of infection was recorded. The bile ducts and gall bladders were opened and measurements were taken. The parasites were fixed in 70% alcohol, embedded in paraffin, sectioned at 5 μ and stained with haematoxylin and eosin (H & E). Tissues were fixed in 10% buffered formalin, sectioned and stained with H & E. The parasites and tissues were examined by light microscopy. For making permanent slides, the flukes were first relaxed in normal saline by vigorous shaking, fixed in 70% alcohol, stained with alcoholic borax carmin and then mounted on canada balsam as described by Sey (1991). The parasites were identified following Sey (1991). The data were analyzed by chi-square method.

RESULTS

Prevalence

The overall prevalence in buffaloes and cattle was 75.07 and 50.79% respectively (Table 1 & 2). Amphistomosis in rumen alone was recorded in 15.62% of buffaloes while in 84.38% of the animals as mixed infection (both in rumen and liver). The respective figures for cattle were 67.71 and 32.29 percent. There was non-significant difference in prevalence during different months of study in both species.

Table 1: Prevalence of amphistomosis in buffaloes during different months of the study period.

Month and year	No. of Animals			
	Examined	Infected		
		Total	Rumen alone	Rumen and liver both
December, 87	59	43(73)	-	43(100)
January, 88	64	50(78)	9(18)	41(82)
February, 88	29	17(59)	5(29)	12(71)
March, 88	35	27(77)	5(19)	22(81)
June, 88	78	63(81)	17(27)	46(73)
July, 88	15	12(80)	2(17)	10(83)
August, 88	50	35(70)	-	35(100)
September, 88	11	9(82)	2(22)	7(78)
Total	341	256(75)	40(16)	216(84)

- No statistical difference ($P > 0.05$) in prevalence was recorded during different months of study.
- Values in parentheses are percentages.

Table 2: Prevalence of amphistomosis in cattle during different months of the study period.

Month and year	No. of Animals			
	Examined	Infected		
		Total	Rumen alone	Rumen and liver both
December, 87	31	9(29)	1(11)	8(89)
January, 88	52	33(63)	25(76)	8(24)
February, 88	43	21(49)	18(86)	3(14)
March, 88	37	20(54)	15(75)	5(25)
June, 88	12	6(50)	3(50)	3(50)
July, 88	2	1(50)	1(100	-
August, 88	6	3(50)	-	3(100)
September, 88	6	3(50)	2(67)	1(33)
Total	189	96(51)	65(68)	31(32)

- No statistical difference ($P>0.05$) in prevalence was recorded during different months of study.
- Values in parentheses are percentages.

When compared across species, the prevalence was significantly higher ($P<0.00001$) in buffaloes as compared to cattle.

Gross Appearance

A: Amphistomosis in Rumen

Depending upon the degree of infection, amphistomes were observed in multiple, dense patches of variable sizes in different parts of infected rumens, especially in the pouch adjacent to the reticulum. These were identified as *Gastrothylax crumenifer*. The flukes were deep red in colour, elongate in shape and measured 8-14 X 4-5 mm in size and were embedded between the villi which were sparse in areas of heavy infection. Hyperplasia of villi was observed in some cases.

B: Amphistomosis in bile ducts

In the affected buffaloes, amphistomes were observed in the common bile duct, cystic duct, hepatic

ducts and in the intrahepatic ducts. The common bile duct was increased in length, diameter and thickness, compared with normal tissue. The average length, diameter and wall thickness of infected bile ducts in buffaloes were 13, 5.6 and 0.4 cm, respectively. The respective figures for normal bile ducts were 4, 1.0 and 0.05 cm. The diameter of infected bile ducts was larger than the duodenum (Fig. 1). Upon opening the infected ducts, they were found to be filled with thick, viscous, yellowish bile and the mucosa was studded with numerous small ovoid parasites. The parasites were firmly attached to the wall and when forcibly removed left small, round (up to 0.2 cm in diameter), white, nodules or buds (Fig. 2). The parasites, identified as *Gigantocotyle explanatum*, were ovoid or conical in shape, 7 to 13 mm in length and 4 to 5 mm in largest diameter and light pink or grayish in colour. In cattle the walls of infected bile ducts were thickened and firm, but there was no increase in their length and diameter. Formation of papillary processes and calcification were commonly seen in the mucosa of infected bile ducts. The infections were lighter and did not extend into intra-hepatic bile ducts.

Histopathological Findings

The histopathological changes appeared to be related to stage of infection. The amphistomes were attached to the walls of bile ducts by drawing plugs of mucosa into their acetabula or posterior suckers. The most prominent change was mucosal hyperplasia. Gradually, the proliferative mucosa formed villi and papillary processes, projecting into the lumen, resembling intestine (Fig. 3). Edema, focal mononuclear cell infiltration and occasional dilated, cystic glands were observed.

The changes in the mucosal plugs, inside the acetabula were different as compared to the rest of mucosa. Inside the acetabula the mucosal glands were at first dilated and hyperplastic (Fig. 4). Later, an increasing amount of inflammatory cell infiltration occurred in the lamina propria, which replaced the mucosal glands. The infiltrating cells included plasma cells, lymphocytes, histiocytes and a few eosinophils and neutrophils. Neutrophils were prominent in the upper areas in contact with the acetabulum, as well as free in the spaces between the mucosa and acetabulum (Fig. 5). Eosinophils were more prominent in cattle as

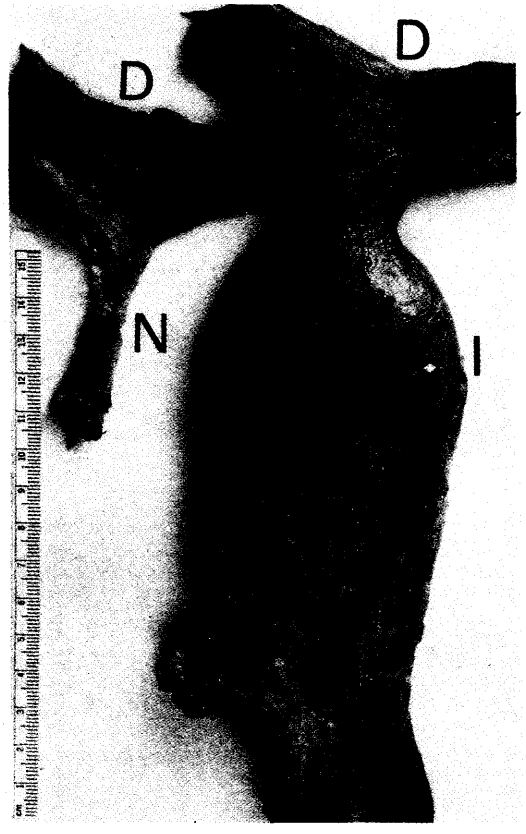

Fig. 1: An enlarged common bile-duct of a buffalo infected with amphistomes (I) compared with normal (N) duodenum (D).

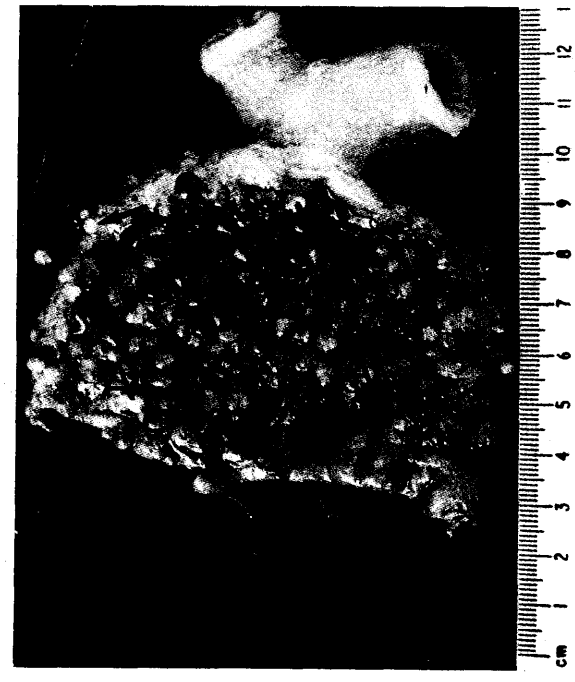

Fig. 2: The wall of a common bile-duct of a buffalo studded with many amphistomes (A). Small buds (arrows) remain after removal of amphistomes.

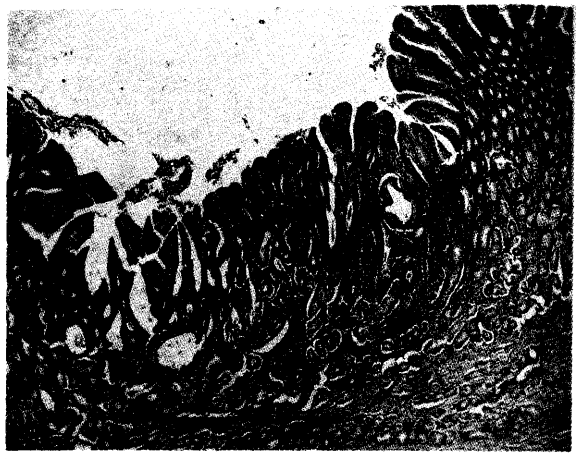

Fig. 3: Chronic amphistomosis in a buffalo. There is hyperplasia of bile-duct mucosa forming villi in the lumen (40 X).

Fig. 4: Chronic amphistomosis in buffaloes. Mucosal plug (bud) with a few distended glands inside the acetatulum (A) of an amphistome (25 X).

compared to buffaloes. In chronic cases only a few glands could be seen in the mucosal plugs. The columnar epithelium at the surface was lost and replaced by bluish amorphous material. Some proteinaceous material was present in space between the mucosa and the acetabula. In the infected rumens, the area of mucosa in contact with the acetabula was covered with a thick layer of parakeratin.

DISCUSSION

Amphistomosis is a widespread disease of buffaloes, cattle, sheep and goats. Acute disease has been reported in young animals while older animals are

Fig. 5: Chronic amphistomosis in buffaloes. Mucosal plug with distended glands and heavy infiltration by inflammatory cells (IC) inside the acetabulum (A) of an amphistome (100 X)

capable of withstanding massive infections (Soulsby, 1982). The existence in Pakistan was reported earlier (Yusuf and Chaudhry 1970; Siddiqui and Shah, 1984; Khan and Anjum, 1994) but information on its seasonal distribution is lacking. The present study was conducted for eight months and a statistically non-significant difference in prevalence was recorded during the different months of study within each species. Heavy infections of paramphistomes both in rumen and liver, are mostly insidious and generally do not cause serious damage to the host (Horak, 1971; Soulsby, 1992). Due to its mild sub-clinical nature, deworming for the control of chronic amphistomis is done very rarely and large animals, once infected carry this trematode throughout rest of their life. Aged animals slaughtered at abattoirs in Pakistan further enhances the chance of acquiring such infections. Statistically non-significant difference in prevalence observed during different months of study may be attributed to the age and lack of deworming practices in large animals. Higher prevalence in buffaloes as compared to cattle might be due to wallowing habit and grazing water grasses and weeds in the former species.

Pronounced hyperplasia of bile ducts in buffaloes with heavy *Gigantocotyle* infection is probably caused by irritation from heavy parasitic loads and subsequent bile retention, because of increased resistance to its flow. The resultant interference with bile excretion into the intestine would adversely affect food digestion and absorption in infected animals. Heavy infections of amphistomes would cause protein depletion,

hypoproteinemia, edema and emaciation and productivity of infected animals would certainly be decreased.

Marked differences in bile duct reactions to amphistomosis were observed between the infected buffaloes and cattle. In infected buffaloes the length and diameter of bile ducts were greatly enlarged while in cattle the tissue reaction consisted primarily of sclerosis, calcification and proliferation of mucosa with the formation of papillary processes. This was probably due to differences in each host's response which has been observed in other infections as well. Sabri *et al.* (1982) compared the responses of buffaloes and cattle to natural fasciolosis and observed that calcification and infiltration by eosinophils was more pronounced in the latter species.

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