INT. J. BIOL. BIOTECH., 6 (1-2): 75-82, 2009.

HISTOPATHOLOGICAL CHANGES IN THE INTESTINE OF *VIPERA RUSSELLI* ASSOCIATED WITH NEMATODE LARVAE

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ABSTRACT

Nematode larvae are the common helminth parasite in vertebrates. They also affect reptiles. The present study deals with the collection of nematode larvae from the intestine of snake *Vipera russelli* and histopathological observations on the infected intestine by nematode parasites. This infection badly affects the structural morphology of the intestinal wall specially the villi. Necrosis, atrophy and degeneration of the villi totally destroy the internal surface microanatomy of the intestine. Further observation from the same material has revealed extrusion of the exudates on to the surface of the bowl. Hemorrhage, inflammation, adenoma, ulceration, atrophy, necrosis, degeneration and erosion of the surface layers was seen in several sections. Numerous large, pink stained inflammatory cells were scattered in the affected areas.

Key Words: *Vipera russelli*, snake, histopathology of intestine, nematode larvae, helminth parasites.

INTRODUCTION

Snake are considered to be a potential source of protein. Economically, their skin is of great commercial value and is used in the preparation of different consumer items such as bags, purses and shoes. Their venom has also medicinal value and meat utilize for the preparation of various food stuffs.

On parasitological aspects, little work has been carried out on snakes throughout the world. Schmitt and Kuntz (1966) find out *Shpaerechinorhynchus serpenticola* (Acanthocephala) from Asian cobra. Le-Van-Hoa and Pham-Ngoc-Khue (1968) worked on parasite of viper. Arevjo (1969) also worked on snake parasites. Akinboade and Dipeolu (1982) worked on a parasite of African snake. Vaucher and Bain (1973) studied the development of parasites in snakes.

In Pakistan, there are few reports available on different aspects of reptiles whereas only three reports are available for the parasites of snake published by Bilqees and Rehana, 1975; Khan, *et al.,* 2002 and Bilqees *et al.,* 2004. The present study deals with the collection of nematode larvae from the intestine of snake *Vipera russelli* and pathological changes due to these larvae.

MATERIALS AND METHODS

Fourteen specimens of Russell's viper (*Vipera russelli*) were collected from interior Sindh and brought to the laboratory of Parasitology Section, Department of Zoology, University of Karachi. Nematode larvae were recovered from the intestine of infected snakes. They were studied under microscope in saline water then preserved in a solution of 5 parts of glycerin and 95 parts of 70% ethyl alcohol.

Infected section of intestine were fixed in 10% Buffer formalin for 24 hours then washed several times with 70% alcohol and left in it. For further processing the tissue were dehydrated in graded series of alcohols then were transferred to cedarwood oil for clearing. Cedarwood oil was removed by placing the tissue in xylol. Slow penetration of wax was made possible by keeping the tissue in a mixture of equal amount of xylol and melted wax, then left in pure wax for overnight. Blocks were made in L-blocks. 6-8 microns thick sections were prepared and parts of section ribbons were kept on slides. Sections were stretched with slight heat of the burner, then kept in xylol for deparafinization. The slides were kept in 50% alcohol than 70% alcohol and stained with hematoxylin and eosin and dehydrated in graded series of alcohols, cleared in clove oil, washed in xylene and mounted permanently in DPX. Photographs of selected portions of the sections of intestine were prepared with a Nikon (Optiphot-2) Photomicroscope using Fuji color film.

RESULTS

Histopathological study of the intestine of a snake *Vipera russelli* parasitized by larvae of *Rhaphidascris* sp. were observed, based on the serial sections of intestine. Study of these sections revealed that the intestinal villi were

badly affected, fused together and have lost their upright position (Figs. 1, 2, 3, 4, 6, 9 and 10). At several places the villi and whole thickness of the muscular layers were distorted, necrotic and inflamed, mostly infiltrated with lymphocytes which appear as blue round dots with H & E stain.

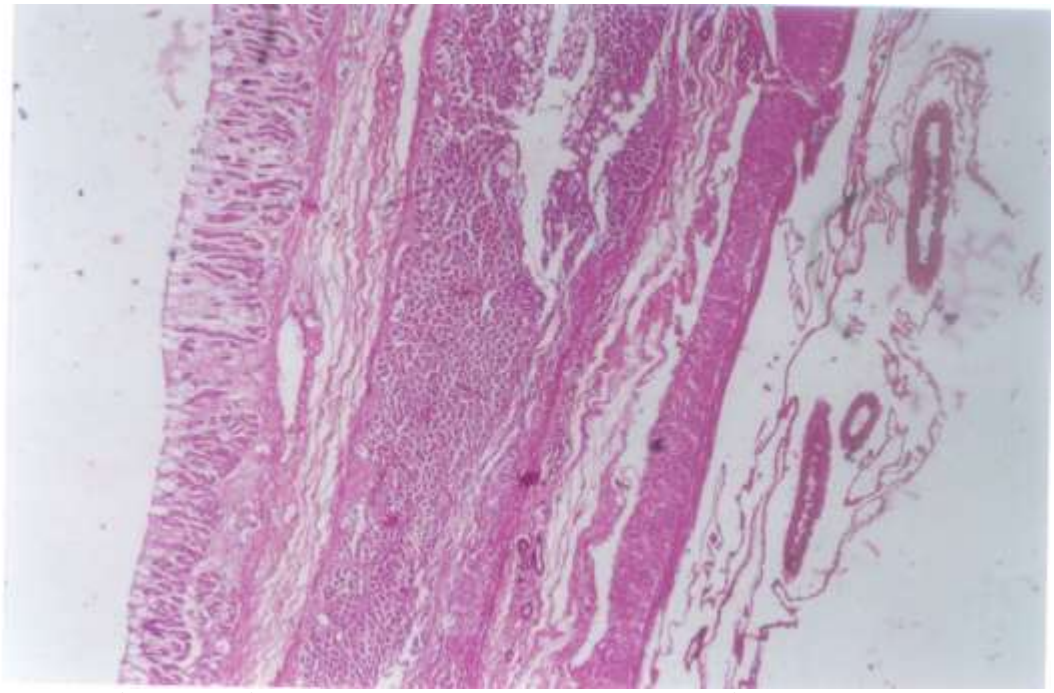

Fig. 1. Section of the portion of intestine showing abnormal mucosal region, marked fibrosis and hypertrophy in the sub-mucosal region, muscularis mucosal region showing degenerated longitudinal and circular muscles, serosa also eroded along with degenerated arteriols X10.

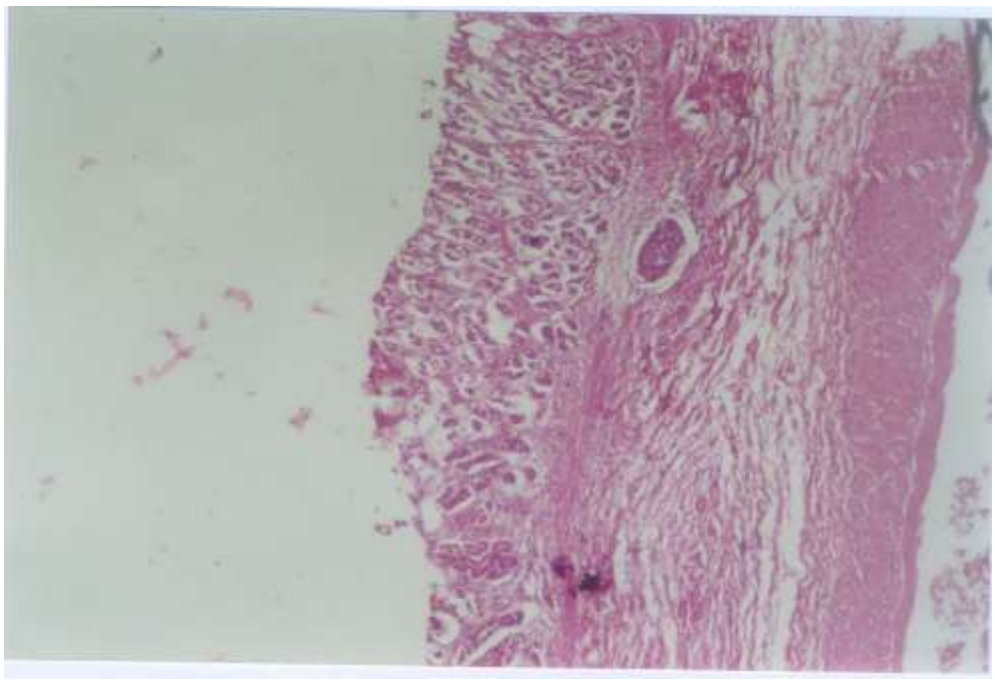

Fig. 2. Section of the portion of intestine showing degenerated nematode larvae in the sub-mucosal region, the atrophic response has distorted the normal architecture X20.

Serosal layers were eroded and in affected regions even where the nematode was not physically present muscular layers have become atrophied with erosion of the adjacent area (Figs. 1, 2, 3, 6 and 10). Entritis

accompanied with total destruction of villi was a common finding. Perforation with large granuloma in the mucosal, sub-mucosal layer was quite obvious in many sections indicating severe host tissue reaction and total destruction of the intestinal tissue (Figs. 1, 2, 3, 4, 5, 7, 8 and 10). These lesions indicated that the nematode has penetrated and perforated intestinal wall. In some of this type of granulomatous lesions, lymphocytes, fibrous elements, several macrophages were present. Large number of fibrous elements were obvious (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10). Total destruction and degeneration was obvious in the muscular and serosal layer. Study of a series of sections has confirmed that whole thickness of the intestinal wall was affected and granulomatous lesions were formed as a result of toxic effect of parasites while perforations were formed at the site of penetration.

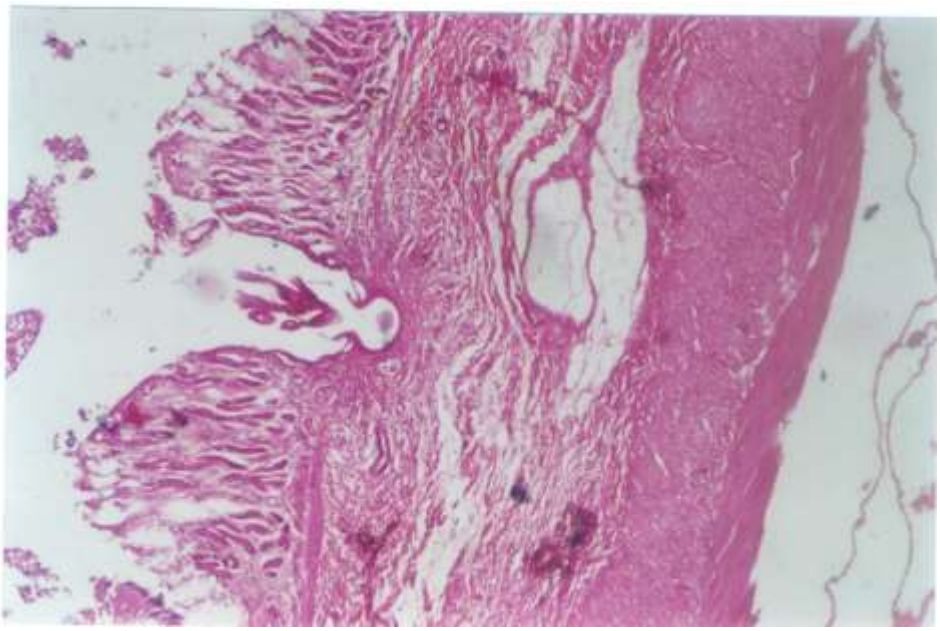

Fig. 3. Section of the portion of intestine showing ulcerative lesion in the mucosal region, abnormal villi are also prominent, lipidosis obvious in the sub-mucosal region X20.

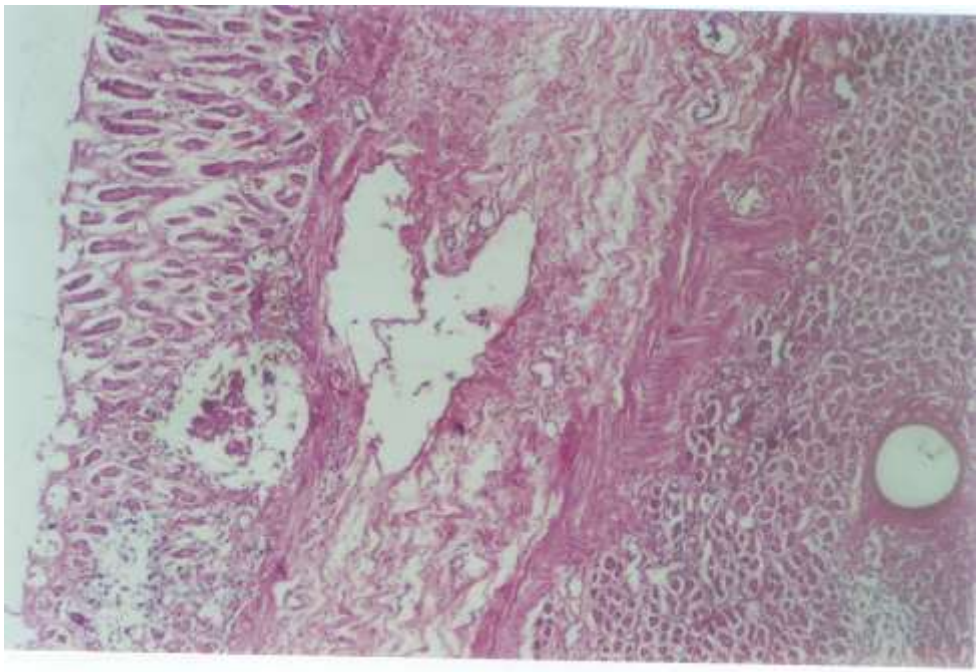

Fig. 4. Section of the portion of intestine showing marked distortion of normal architecture of intestine. Hypertrophy and necrotic foci in the muscularis mucosal region, fibrosis in the sub-mucosal region. Fusion and enlargement of gland cell in the mucosal region is obvious, a nematode larval tunnel (clear space) is also present in the mucosal region on the right lower portion (arrow) X20.

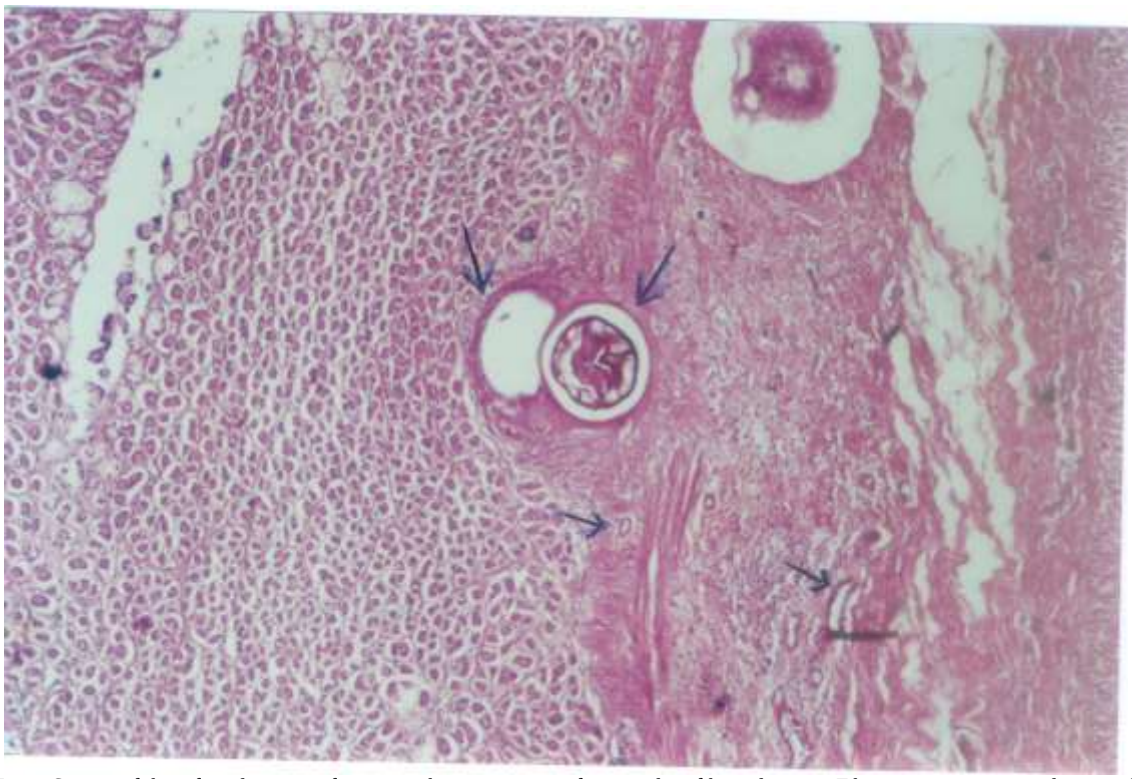

Fig. 5. Section of the infected portion of intestine showing section of nematodes of buccal region. Fibrosis is present around nematode larvae and around the clear space (arrow), degenerated venules (small arrow) also prominent in the vicinity of nematode larvae, Atrophy in the mucosal region is obvious X20.

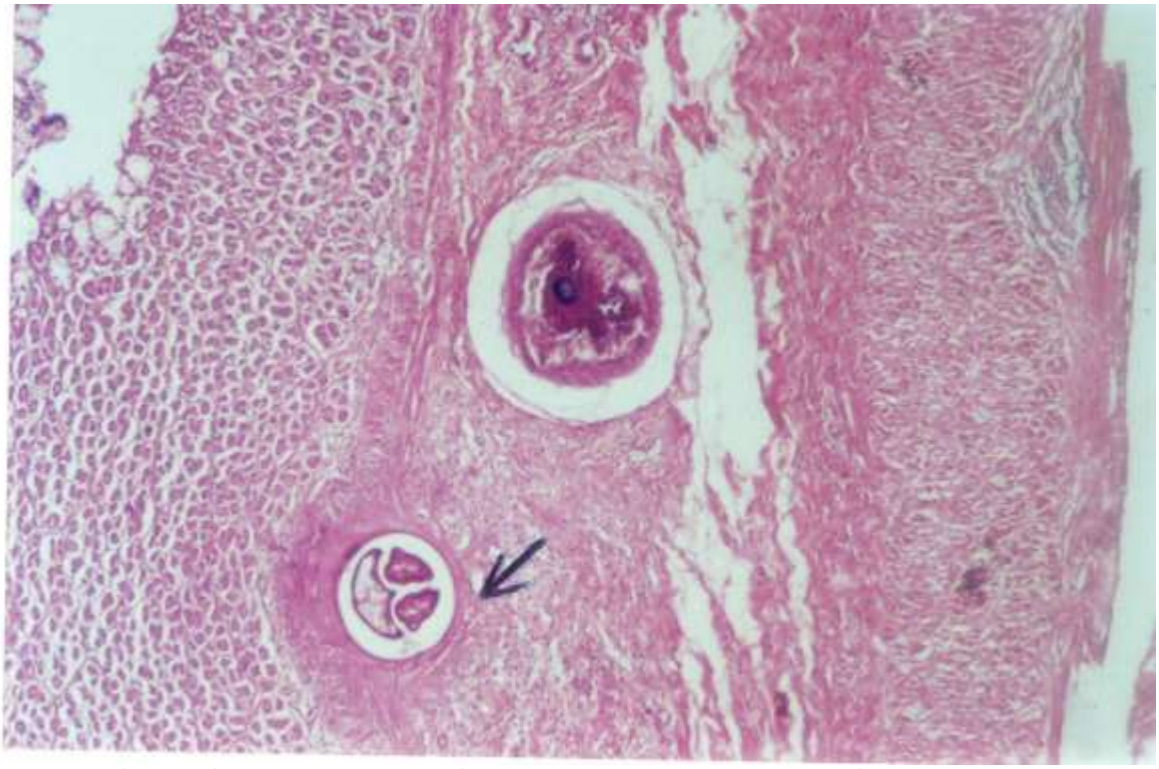

Fig. 6. Section of portion of intestine showing section of nematodes, three lips are prominent in one of the nematode section (arrow) marked atrophy in the mucosal region on the left is much obvious X50.

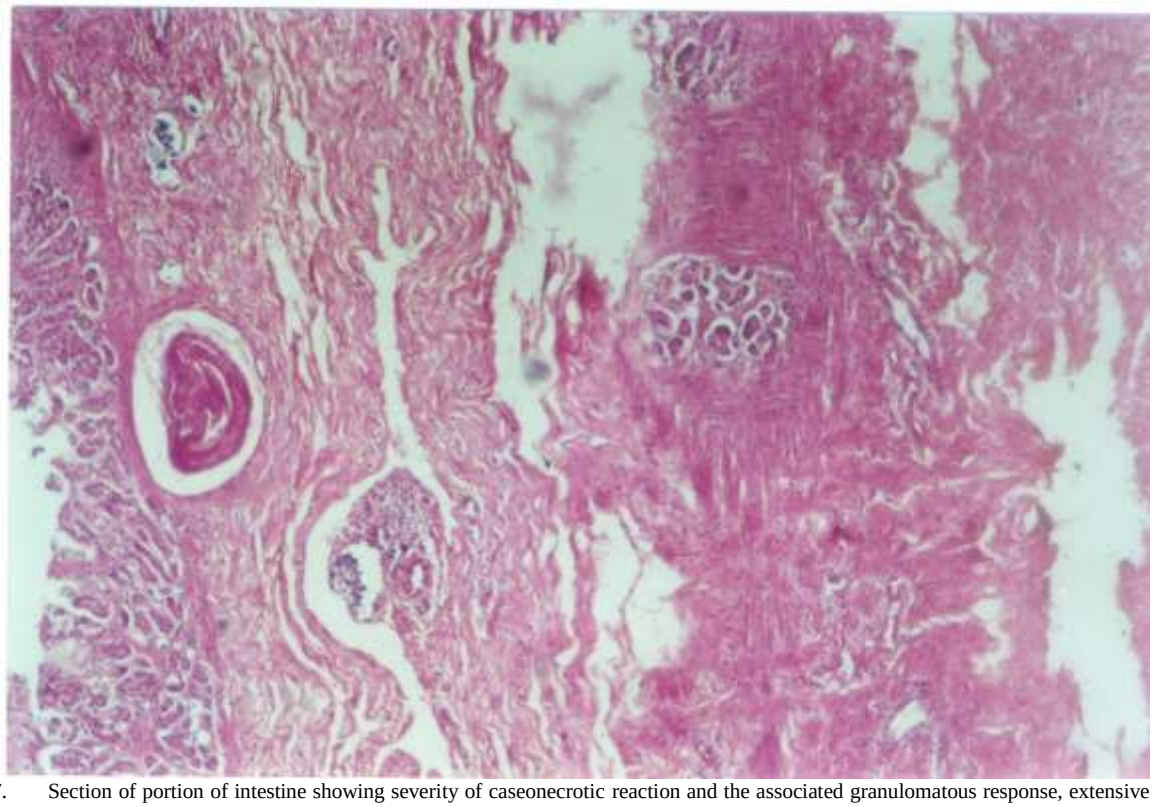

Fig. 7. Section of portion of intestine showing severity of caseonecrotic reaction and the associated granulomatous response, extensive areas of fibrosis along the section of nematode is obvious X20.

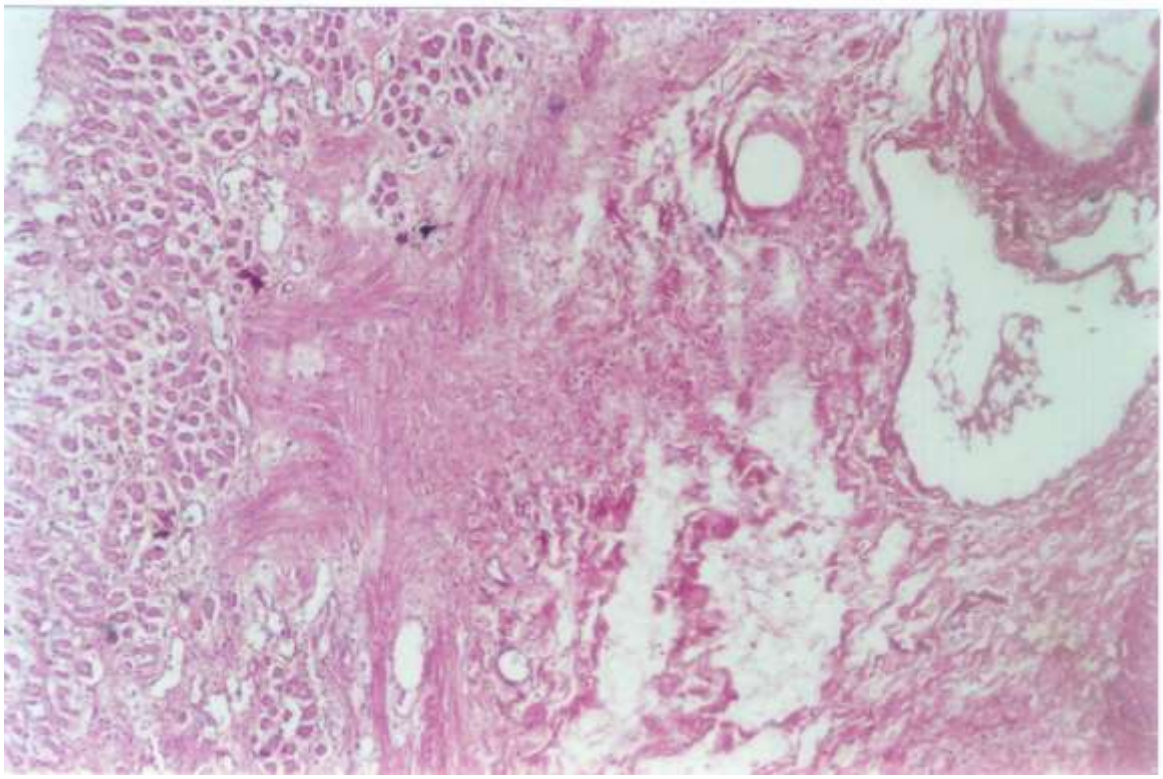

Fig. 8. Section of portion of intestine showing caseous necrosis and lipidosis X20.

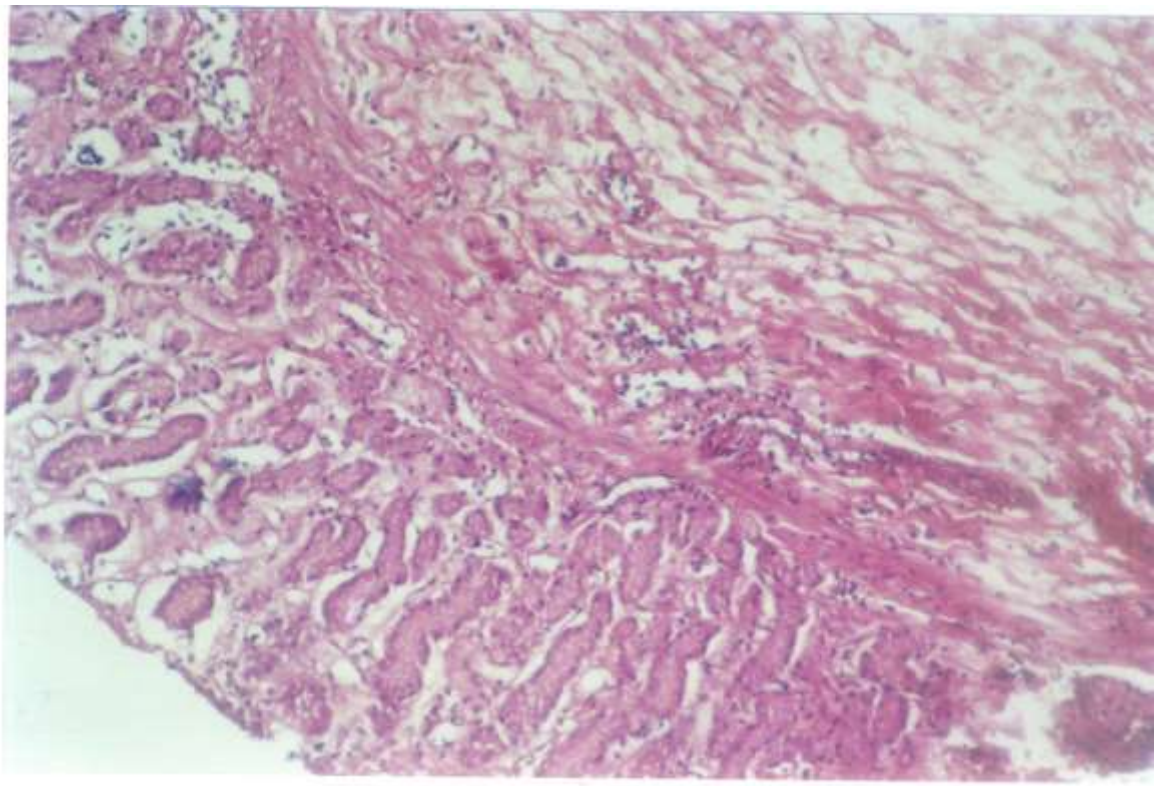

Fig. 9. Section of portion of mucosal region of intestine showing fusion and atrophy of gland cell, abnormal and necrotic lamina propria and atrophic sub-mucosal region X20.

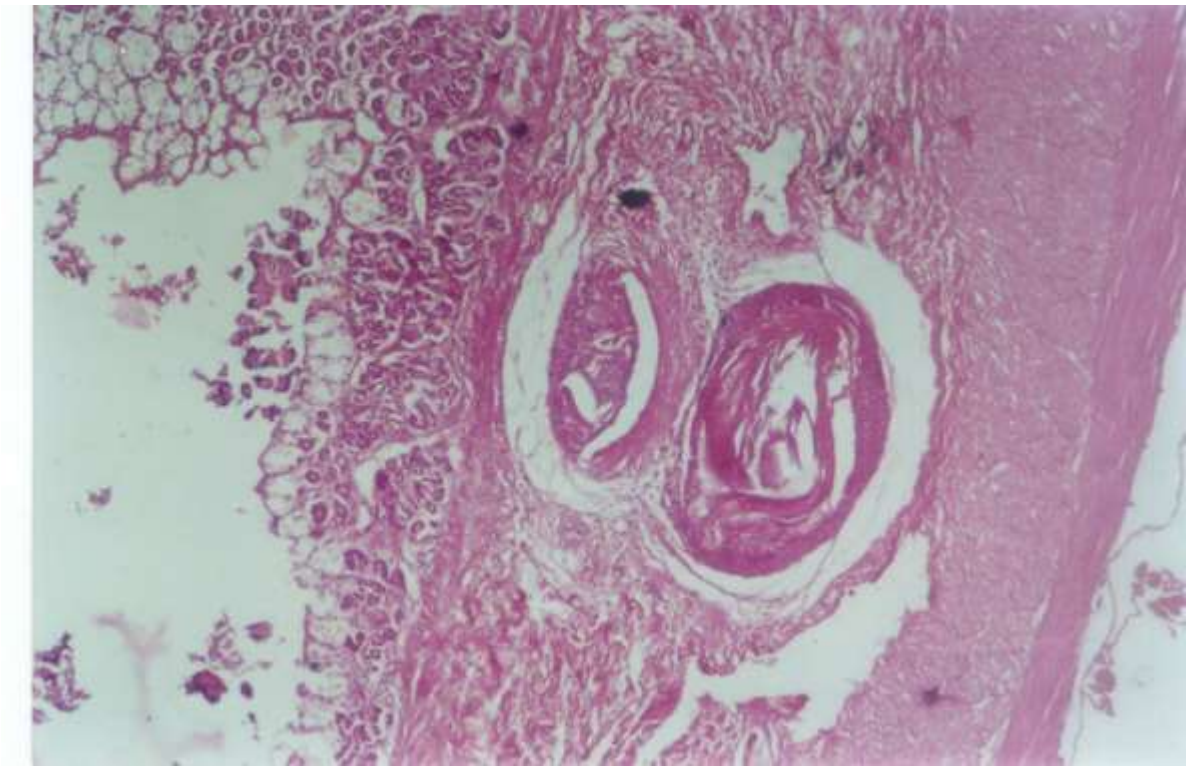

Fig. 10. Section of the portion of intestine showing sub-mucosal granulomas with liponecrotic cores X20.

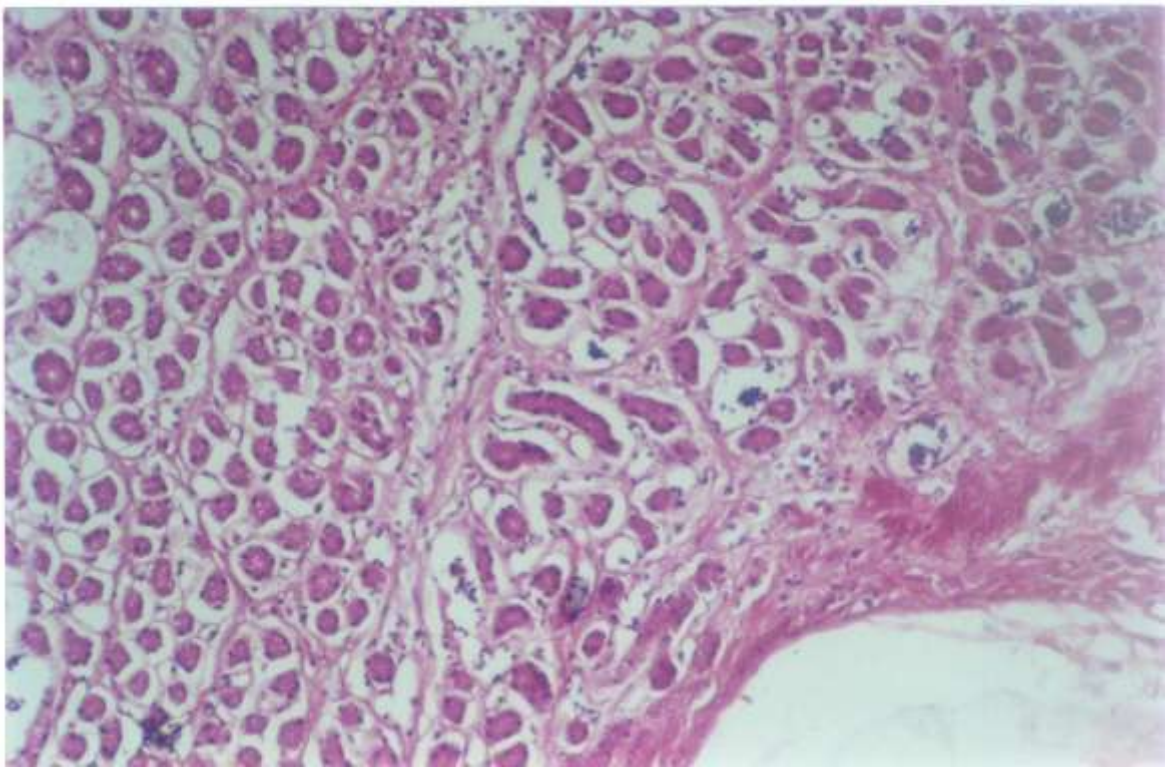

Fig. 11. Section of the portion of intestine showing honey-comb like portion of sub-mucosal region indicates fibrosis, atrophy and early stage of necrosis. Inflammatory cells also infiltrated in the area X20.

Fusion of villi and degeneration of epithelial cells was prominent (Figs. 1,2, 3, 4 and11). The serora was eroded. No parasite tissue was obvious in this granuloma. The muscularis mucosa or the muscles layers lost their structure and converted into fibrous mass. The serosal region also atrophied and eroded along with abnormal arterioles (Fig. 1). In some sections marked hypertrophy obvious in sub-mucosal region (Fig. 11). Another important finding is the presence of ulcerative lesion at the mucosal region (Fig. 3). At places where section of nematode present were surrounded by large number of macrophages and fibrinous exudates (Figs. 5, 6 and 7).

DISCUSSION

Generally granulomatous inflammatory disorders in the gastrointestinal tract are referred as enteritis. This may involve any portions of the gastrointestinal tract. The granulomas represent building up of cell mediated immunity to the causative agent (Spector, 1969). As per details given by Spector (1969) the initial stage neutrophils and macrophages aggregate at the site of infections. The macrophages engulf the necrotic tissue and dead cells. This is the development of cell mediated immunity, leading to the generation of specifically sensitized, lymphocytes. These lymphocytes then accumulate at the site of infection, liberate several soluble factors, some of which attract, immobilized and activate more macrophages. At the some time, the macrophages undergo epitheloid cell transformation. It appear that the granulomatous lesions observed in the present studies in intestine of snake initially was an effort to produce cellular immunity in which the host tissue has not succeeded due to the full thickness involvement at the intestinal wall. The granulomatous lesions appear severe and irrepiarable.

It is likely to suggest that due to full-thickness enteritis the host might have faced nutritional problems due to lack of absorption of the nutrients from the lumen of the host intestine. As the villi are totally damaged and where they remain attached as in (Figs. 1,2, 4 and 9) are fused together, the underlying tissue with granulomatous lesions and alteration in muscular layers which have became homogeneous appear to disturb function of the organ.

It is concluded that the severe destruction of intestinal wall is irreversible. This might have resulted into malabsortion and can be fatal to the host.

As the snakes are utilized to prepare several dishes in different countries of the world so the heavily infected snake is obviously not desirable for human consumption as the secretary and exeretory products from the parasite may have antigenic effect, may produce digestive symptoms and allergic reaction as do the other parasites and larval nematodes (Moreno-Ancillo *et al*., 1997).

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(*Accepted for publication January 2009*)