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GASTRO-INTESTINAL HELMINTHIASIS: AN UNSEEN THREAT TO THE BACKYARD POULTRY PRODUCTION OF KASHMIR VALLEY

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ABSTRACT
The present investigation was carried out to have sound background information about the damage caused by the gastrointestinal helminthes to the backyard poultry production which has been a traditional component of small farms throughout the developing world (Branckaert, 1995). The helminthes were identified and classified into 3 groups, according to their taxonomy. Trematodes (Intestinal flukes) comprised of *Echinostoma revolutum* (7.9%), *Notocotylus attenuatus* (1.2%) *and Paramnostomum galli* (0.2%). Cestodes (tapeworms) were *Amoebotaenia sphenoides* (6.69%), *Raillietina* tetragona (36.19%), *Raillietina cesticillus* (23.2%) & *Choanotaenia infundibulum* (20.9%). The last category was nematodes (roundworms), which comprised of *Ascaridia galli* (35.35%), *Heterakis gallinarum* (4.39%), *Capillaria anatis* (8.78%) and *Acuaria hamulosa* (3.5%). The intestinal flukes were found to inhabit small intestine attached to the intestinal mucosa. Tapeworms were found especially inhabiting duodenum and jejunum parts of the intestine attached to the mucosa with the help of rostellum and the nematodes were found to be lying either free in the lumen of the intestine or within the musculature of gizzard. In general histological examinations of the infected tissue revealed mucous degeneration with vacuolation of lining epithelial cells, mucous enteritis, broadening of the villi. The intestinal wall appeared to be thickened with mucosa giving a velvety appearance. Histological studies of the infected mucosa presented multiple erosion foci together with parasite and cellular debris. Parasite sections and eggs were observed in the lumen which was full of cellular debris. Cellular reaction was diffuse in the mucosa and submucosa and was characterized by large number of lymphocytes, monocytes, plasma cells, heterophils and few eosinophils. The mucosa and submucosa showed marked thickened and diffuse mononuclear infiltration and also reactive lymphoid nodules.

KEY WORDS- Helminthiasis, threat, backyard poultry, Kashmir.

INTRODUCTION

Backyard poultry is popular among rural people providing supplementary food, extra income and employment for them. But the poultry production is hindered by many problems among which various diseases namely bacterial, viral and parasitic infections are the most important (Ojok, 1993). In fact the free ranging backyard chickens feed on a wide range of food substances ranging from grains, fruits to insects which may harbour infective stages of parasites thereby predisposing them to parasitic infection particularly gastro-intestinal parasites (Oniye *et al*., 2001; Frantovo, 2000). The occurrence and the pathological changes associated with the gastro-intestinal helminthes has been very well reported (Fatihu *et al.*, 1991; Khan *et al*., 1994; Ibrahim *et al*., 1995; Amin-Babjee *et al*., 1997; Salam *et al*., 2009; Salam *et al*., 2010). Endoparasites dilate the intestine, produce nodules and severe enteritis, thus impairing the absorbing power of intestine for nutrients and vitamins from the host. The resultant situation leads to loss of body weight, retarded growth, reduced egg production, weakened body resistance and even death (Hayat and Hayat, 1983). The aim of this research therefore was to investigate various aspects of helminth infections in poultry with the hope of collecting base line data so that further research could be conducted on the control measures that will ultimately help reduce the suspected high prevalence of helminthes and thus increase the productivity of the poultry industry in general.

MATERIALS & METHODS

This study was conducted for a period of two years from January 2011 to December 2012 on a sample size of 478 semi-scavenging backyard chickens collected from different localities of Kashmir to determine the prevalence of gastrointestinal helminth infections in our backyard poultry and also have an idea of the extent of the tissue pathologies produced by these helminthes. After collection, each of the gastrointestinal tracts was examined thoroughly from the outer surface, to detect the gross pathological changes, if any. Therefore, the gastrointestinal tract was subjected to routine examination to collect the gastrointestinal parasites, according to the procedure as described by Fowler (1990). Internal surface of the intestinal tracts was also investigated thoroughly to detect the gross pathological changes, if any. From the suspected viscera, mucosal scraping was taken and examined under microscope at 10X magnification by adding a drop of normal saline, mounting with a cover slip to detect tiny parasites which deeply burrow into the mucosa, if any. Parasites of the intestinal tract were separated from the intestinal content by repeated sedimentation and made clear by gentle washing with PBS. After washing, nematodes were collected by the help of curved needle and

kept in glycerin alcohol. Cestodes and trematodes were collected by the help of dropper and preserved in 10% formalin for the identification. Thorough morphological study of nematodes was performed by the preparation of sub-permanent slide by adding one drop of lactophenol. Morphology of trematodes and cestodes were studied by preparing permanent slide according the methods as described by Cable (1957). Parasites were identified according to the keys and description given by Soulsby (1982) and Yamaguti (1958). The intestines showing gross abnormalities were fixed in 2.5% glutaraldehyde for Scanning Electron Microscopic studies and for histological studies the infected intestinal tissues were fixed and preserved in 10% formalin, processed through conventional technique (Behmer *et al*., 1976) for paraffin embedding and then sectioned at 4-5 microns in thickness. The sections were then stained with Haematoxyline and Eosin). Prevalence was calculated as a percentage of the host population infected at a point in time (Thrusfield, 1995). Mean intensity was calculated as number of parasites per infested bird.

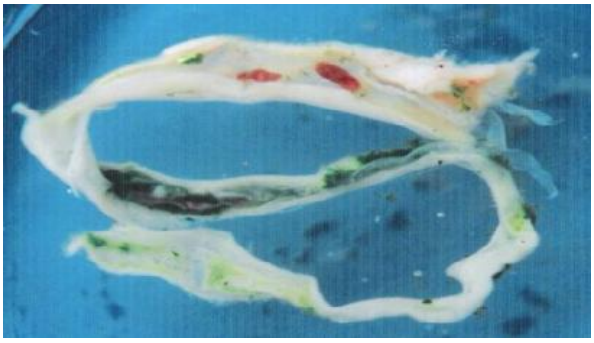
RESULTS

After examining the viscera of 478 domestic chickens, the various helminthes recovered were classified into 3 groups, according to their taxonomy. Trematodes (Intestinal flukes) comprised of *Echinostoma revolutum* (7.9%), *Notocotylus attenuatus* (1.2%) *and Paramnostomum galli* (0.2%). Cestodes (tapeworms) were *Amoebotaenia sphenoides* (6.69%), *Raillietina* tetragona (36.19%), *Raillietina cesticillus* (23.2%) & *Choanotaenia infundibulum* (20.9%). The last category was nematodes (roundworms), which comprised of *Ascaridia galli* (35.35%), *Heterakis gallinarum* (4.39%) and *Acuaria hamulosa* (3.5%). The parasitic prevalence was recorded monthly over a period of two years. In general out of a total of 478 birds screened, 205 (42.8%) were found to be infected including 102 of 233 (43.7%) and 103 of 245 (42.04%) during the 1st and 2nd year of study respectively. The prevalence rates varied during different months of the year from 22% (5/22) to 75% (15/20). The mixed infection rates varied from 13.6% (3/22) to 73.6% (14/19). The parasite load and mean intensity of infections for the helminthes were also recorded. Although the highest parasitic load in terms of number of total parasites recovered were found to be associated with *Raillietina tetragona*, yet mean intensity of infection was observed to be highest for *Heterakis gallinarum* (20 ± 2.1) followed by *Amoebotaenia sphenoides* (09±2.2), *Acuaria hamulosa* (08 ± 2.2), *Raillietina tetragona* (06 ± 1.3), *Ascaridia galli* (05 ± 1.9), *Echinostoma revolutum* (03±1.8), *Choanotaenia infundibulum* (03 ± 2.4), *Notocotylus attenuatus* (02 ± 1.8), *Raillietina cesticillus* (02 ± 2.1) and *Paramnotomum galli* (01±1.6). In general the gastrointestinal helminthes were found to be highly prevalent during the summer and autumn and least prevalent during winter. The trematodes were found to inhabit small intestine where they remain firmly attached to the mucosal lining by their suckers (Figure 1). The histological studies of the infected tissues revealed mucosal degeneration, broadening of the villi and sparse inflammatory infiltrations especially of mononuclear cells and few eosinophils.

Cestodes were found especially inhabiting duodenum and jejunum parts of the intestine. In general parasites were found attached to the mucosa with the help of rostellum. Scanning electron micrograph revealed penetration of scolex of *Raillietina tetragona* deep into the mucosa and the mucosa appeared to be rough (Figure 2). Histopathological examinations revealed broadening of villi which at places were fused to give much broader appearance. Scolices of the parasite were found embedded in the superficial mucosa. The mucosa revealed mechanical disintegration of the lining epithelium and glands in the vicinity of the scolices. Inflammatory reaction around the lesions was characterized by lymphocytes and a few heterophils (Figure 3). Infection with *Raillietina cesticillus* was characterized by the presence of robust cestode anchored to the mucosa with the help of scolex. Scanning electron micrograph revealed scolex of the parasite embedded deep into the mucosa and the intestinal villi were flattened (Figure 4). Histological study revealed the sections of the cestode in the lumen associated with the atrophy and broadening of the intestinal villi. The epithelium and glands at the site of infection were disintegrated. The inflammatory reaction was characterized by predominant heterophils, especially in the areas of mechanical damage by scolices. Also a few lymphocytes and eosinophis were seen, especially in the lamina propria (Figure 5). In the fowl intestines infected with *Amoebotaenia sphenoides*, SEM revealed that the parasite was firmly attached and deeply embedded into the mucosa of duodenum (Figure 6). Also, a few parasites were found free into the lumen of lower jejunum. Histopathological sections revealed the scolices burrowing deep into the mucosa, with the mucosal plug extending into the sucker cavity. In general villi appeared to be atrophied with rounding of tips. At the site of infection, the epithelium and glands were disintegrated. The inflammatory reaction was characterized by mononuclear cell infiltration which was more severe at the site of infection (Figure 7). The degree of tissue damage as well as cellular infiltration was more severe in case of mixed infection of *Amoebotaenia sphenoides* and *Choanotaenia infundibulum*. Mixed infection of the two cestodes was characterized by atrophy of villi with rounding of tips, vascular congestion, haemorrhages in the mucosa and cellular infiltration involving mucosa and submucosa (Figure 8).

Among nematodes, *Ascaridia galli* were found to inhabit lumen of the intestine. Gross changes observed depended on the parasite load. In most of the cases, low load of worms was observed and was not associated with any grossly observable lesions. Moderate infection was associated with mucous enteritis. The intestinal wall appeared to be thickened with mucosa giving a velvety appearance. Lumen contained thick white pasty mucous. Histopathological sections of the parasites were found in the lumen (Figure 9). The histopathological lesions varied from degeneration of lining epithelium to sloughing of mucosa. Mucous degeneration with vacuolation of lining epithelial cells was a consistent feature. Cellular reaction was mild and was characterized mainly by mononuclear cells and a few polymorphonuclear cells including eosinophils. At places local mononuclear infiltration was observed in muscle layer (Figure 10). *Heterakis gallinarum* occurred free in the lumen of caecum especially at the

blind end. The caecal wall was thickened and revealed petecial haemorrhages. Histopathologically mucosa revealed degeneration of mucosa and glandular distortion. Parasite sections and eggs were observed in the lumen which was full of cellular debris (Figure 11). Cellular reaction was diffuse in the mucosa and submucosa and was characterized by leukocytes and heterophils. Nodular aggregation of mononuclear cells was also observed. Toludine blue stained sections revealed presence of mast cells in the submucosa and mucosa (Figure 12). *Acuaria hamulosa* worms were found deeply embedded in the musculature of the caudal lobe of the gizzard (Figure 13). The parasites could be detected after the removal of Koilin layer and cutting through the musculature. Grossly the musculature of the caudal lobe of the gizzard revealed soft yellowish nodules. Histopathology revealed discrete nodular lesions in the musculature which contained sections of the parasite. Cellular reaction in the lesions was characterized by large number of lymphocytes, monocytes, plasma cells, heterophils. The cellular reaction was either diffuse or densely concentrating around the cyst (Figure 14). At places aggregation of lymphocytes appeared in the form of follicles. In some of the sections severe eosinophilic reaction was observed.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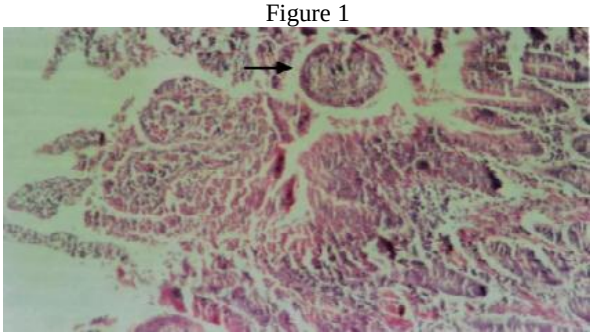
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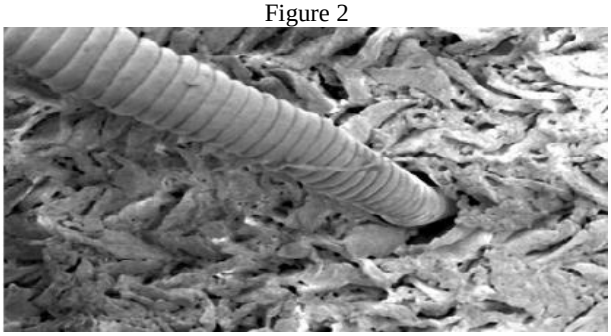
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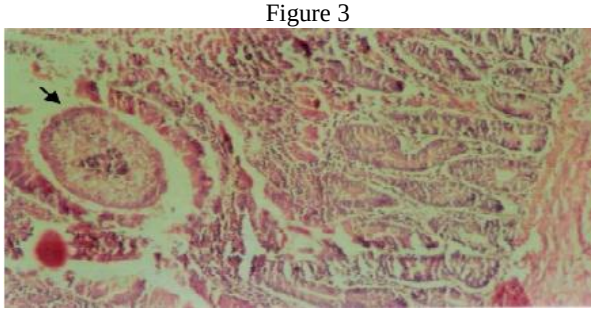
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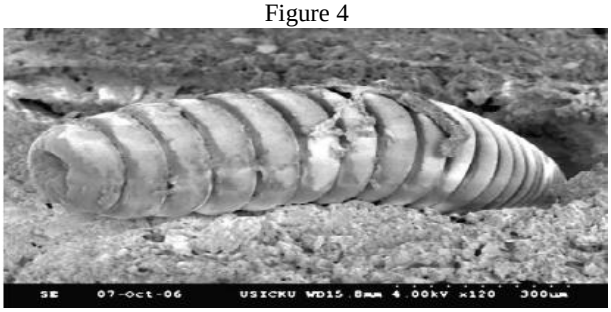

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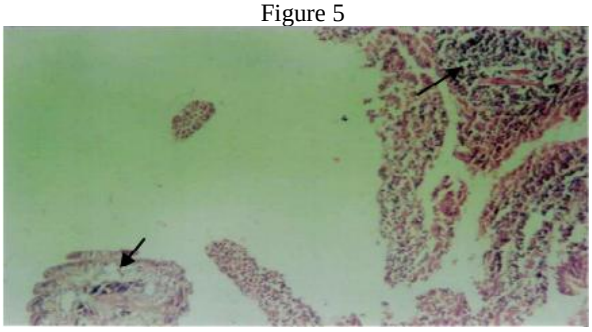
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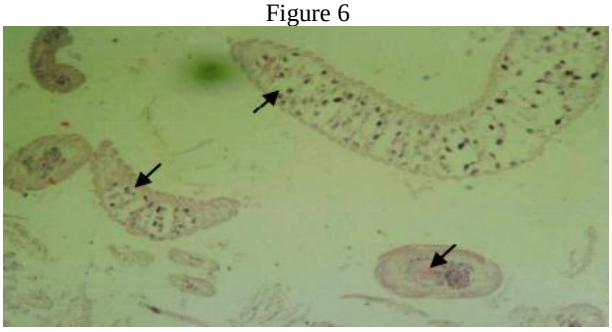
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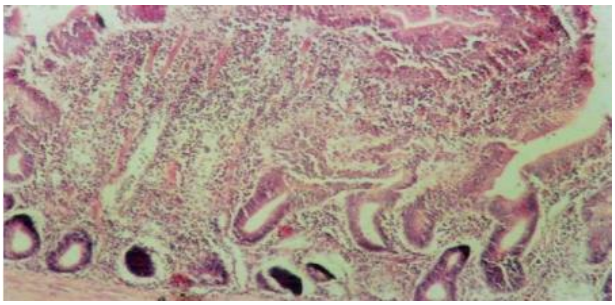

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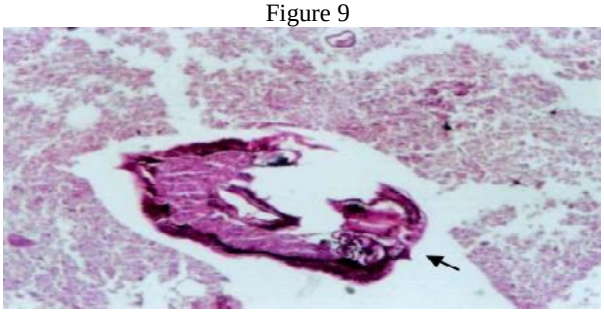

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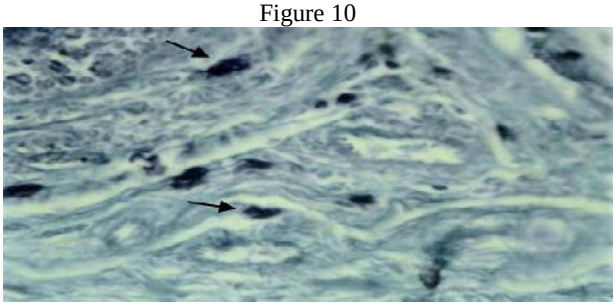

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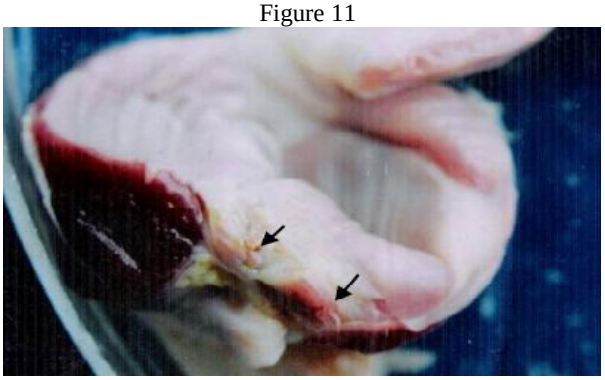

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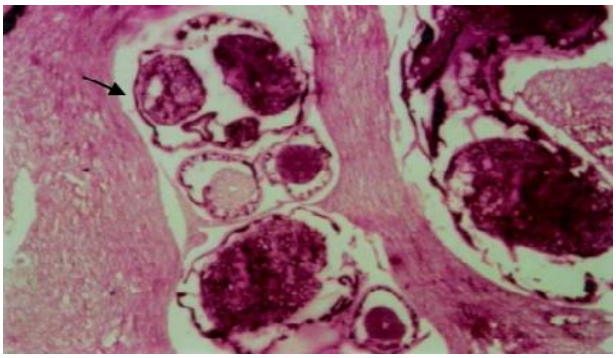

Figure 14

DISCUSSION

In the current study 43% of domestic fowl examined were found to be infected with the endoparasites. Most of the birds were found to harbour more than one species of endoparasites and the overall mixed infection rates in domestic fowl was found to be 32%. In general the overall prevalence rates were found to show a positive correlation with the increase in temperature and humidity. Among the trematodes recovered in the current study, *Echinostoma revolutum* showing an overall prevalence of 7.9% was found to be prevalent throughout the year. Other trematodes like *Notocotylus attenuates* and *Paramnostomum galli* recovered from domestic fowl were observed with very low prevalence during summer and autumn months. *Echinostoma revolutum* has been previously reported from domestic fowl in Kashmir (Pandit *et al.*, 1991). Nithiuthai, *et al.* (2003) reported *Echinostoma* and *Notocotylus* infection in native chicken from Bangkok with prevalence rate of 1% each. Among cestodes, *Raillietina tetragona* was found to be most prevalent with an overall prevalence rate of 36%. Besides *R. tetragona*, *Amoebotaenia sphenoides*, *Raillietina cesticillus* and *Choanotaenia infundibulum* could be recovered from the domestic fowl but with significantly lower prevalence rates. Domestic fowl were found to harbour various cestodes throughout the year. Amongnematodes, chickens were found to harbour *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria anatis* and *Acuaria hamulosa*. Prevalence rates were highest during summer. *Ascaridia galli* was found to be prevalent throughout the year. Other nematodes in general were infrequently recovered. The studies of parasite load in general revealed a low level of infestation in the birds with individual exceptions. However mean intensity of infection for all the three types of parasites were higher in summer months and lowest/nil in the winter months of the study. The observations recorded in the current study are in agreement with the reports of various workers from India and outside. Pandit *et al.* (1991) while working on the prevalence of helminth parasites in local fowls of Kashmir got almost similar percent age prevalence of the helminths. Our results are also coinciding with the observations of Eshetu *et al.* (2001) who also reported that in chicks of Amhara region of Ethiopia, among cestodes, *Raillietina tetragona* and among nematodes *Ascaridia galli* showed the highest percentage of prevalence. Coinciding with our study are the observations of Hassouni & Belghyti (2006) in Morocco, Permin *et al.* (1999) in Denmark, Ashenafi & Eshetu (2004) in Ethiopia and Nithiuthai *et al*. (2003) in Bangkok. Minor differences in the results of the present study could be explained on the basis of seasonal, manage mental, climatic variations and also due to variation in parasitic population of different localities where the birds

were exposed. Our observations are also clearly in line with the observations of Fotedar and Khateeb (1986) who also recorded the highest incidence of helminth infection in the month of September and lowest in the months of December and January and a decrease in the incidence and mean worm burden with decreasing temperature and rainfall. The reason behind the heavy infection during the warm and wet months may be high mean temperature and high relative humidity which lowers the resistance of birds and favours heavy infection (Hawkins, 1945) and lower rate of infection during winter season might be attributed to low temperature which also may help in arrested development of parasites in host and environment (Ogunsui and Eyskey, 1979).The increased availability of intermediate hosts in the rainy seasons for the completion of life cycles of parasites may also be one important factor responsible for high rate of infection during summer months. In current study, pathological effects of endoparasites varied with the load and nature of infection. In general low level solitary infections were not associated with any pathomorphological alteration, while most severe changes could be observed in cases with mixed infection. In general pathogenicity of cestodes in poultry has been essentially incriminated to adult worms and the effects have been found to vary with the type of parasite, parasitic load and host factors (Mir *et al.*, 2003). *Raillietina tetragona* infection was found to be associated with the penetration of the scolices only upto superficial mucosa. Jha *et al.* (1981) reported that suckers reaching up to muscular layer. Also various authors have reported much severe lesions including nodule formation, closely resembling to tuberculosis lesions (Ackert and Case, 1938). In the present study we could also observe such lesions associated with *Raillietina echinobothrida* infection in pigeons. Nath and Pande (1963) also reported that scolices of *Raillietina tetragona* were not found to burrow deeper beyond the mucosa. The observed discrepancies in the severity of lesions could be attributed to low parasite load as recorded in the present study. The lesions observed in the present study were indicative of mechanical damage due to sucker armature. It has been reported that sucker hooks, inserted on the mucosal tissue, exercised a prominent point in affecting a strong hold on the lining than the rostellar hooks which seemed to have a secondary role in attachment (Nath and Pande, 1963). The cellular reaction observed in the present study, although was mild but is in accord with earlier reports (Nath and Pande, 1963; Jha *et al.*, 1981). *Raillietina cesticillus* infection as observed in the present study was characterized by villous atrophy and fusion, increased vascularity and glandular disintegration of varying degree. Similar changes but of varying degree have been reported by other workers (Biester and Schwarte, 1957; Nath and Pande, 1963; Jha *et al.*, 1981). The mucosal changes have been attributed to the mechanical damage. The infiltration of neutrophils around the mucosal lesions, as observed in the present study has also been reported by Nath and Pande (1963). Some authors reported mononuclear infiltration of lamina propria also (Gray, 1975; Jha *et al.*, 1981, Bhowmik *et al.*, 1982). Jha *et al.* (1981) opined that heterophilic infiltration was not a primary reaction but only secondary to bacterial invasion of the mucosal lesions. Infection with *Choanotaenia infundibulum* was associated with mild degree of catarrhal enteritis which may be correlated with the low parasite load.

The pathological changes observed with *Amoebataenia sphenoides* infection in fowl is in agreement with earlier reports (Nath and Pande, 1963; Jha *et al.*, 1981). The parasites have been frequently reported free in the lumen and also attached to the lining. The mucosal damage has been attributed to dragging of epithelial tissue by the parasite sucker. Among the nematodes *Ascaridia galli* were mostly recovered from the lumen of small intestine without causing much gross pathology. However, heavy infections have been reported to cause severe changes. The pathomorphological changes are in agreement with the earlier observations with comparable parasite load (Padhi *et al.*, 1987; Arunachalam *et al.*, 2003). *Heterakis gallinarum* has been considered of low pathogenicity in single infections (Lund and Chute, 1973). However more severe pathology has been associated with the parasite in domestic galliformes, causing nodular typhilitis, with the formation of inflammatory or granulomatous caecal nodules (Kaushik and Sharma Deorani 1969**;** Riddel and Gajadhar, 1988; Khan *et al.*, 1994). Although in the present study, gross nodular lesions were not evident, histopathology revealed nodular aggregation of mononuclear cells as well as parasitic sections in the mucosa. Erosive lesions as seen in the present study have also been recorded by Nath (1961) who opined that eroding habit of *Heterakis gallinarum* imparts greater pathogenic significance to this nematode. The pathological changes associated with the infection of *Acuaria hamulosa* as observed in the current study were very severe and in accordance with the earlier pathological descriptions by Srivastava (1939), Blazekovic (1960), Whur (1966), Nath and Pande (1963), Padhi *et al.*, (1987) and Salam *et al.*, (2009) who recorded the formation of soft nodules inside the musculature of the caudal lobe of the gizzard and destruction of the Koilin layer in heavy infection. However in the present study, the mononuclear infiltration was more severe and formed follicular aggregates. Severe eosinophilic reaction and mucosal changes were additionally recognized. The generalized and striking pathomorphological alterations observed in various intestinal helminthiasis was villous atrophy with fusion and broadening of villi and degeneration of lining epithelium. Such changes interfere with nutrient absorption (Read and Simmons, 1963; Nadakal and Nair, 1979). Also such changes result in loss of fluids and electrolytes. Such changes have a severe reflection on overall health status of the birds and lead to drop in the production as well as increased susceptibility to secondary infections (Mir *et al.*, 2003). Further in most of the cases, the severity of pathological changes was dependent on the parasite load besides the pathogenic potential of the parasite.

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