Histopathological studies of cestodiasis in domestic fowl, Gallus gallus domesticus

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Abstract
Despite great contributions of the livestock sector to the economy and livelihood of the people, the livestock sector is constrained by many challenges especially helminth infestation. Cestodiasis which substantially affects the poultry productivity is caused by digenetic cestodes. Cestodiasis has debilitating effects on the host and proves to be one of the constraints for poultry husbandry. Histopathological studies revealed that the intestine of infected fowl showed haemorrhages, less growth of villi, ulcerations, incisions, nodular growth, eroded mucosal epithelium and load dependent mild to moderate enteritis. In general, the degree and extent of enteric changes corresponded to the parasitic load. Attachment of the parasite caused traumatic lesions which might have favoured secondary bacterial infection and hence more severe cellular reaction in the infected area was found. However delineation of local effects and species specific studies are needed. The most heavily infected segment of the intestine was the lower small intestine followed by the duodenum. The least populated segment was the rectum.

The infected hosts were found weak with less weight, down carrying feathers, ruffled plumage and increased appetite. Most of the infected hosts were infected by only one cestode species. This may be due to the immunity provided by one species against other species. The higher prevalence of the parasites and observed pathology directly reflects their economic importance and warrants conscious intervention for its control in backyard poultry.

Introduction
The domestic fowl, Gallus gallus domesticus, derives its ancestry from the wild Indian and S.E Asian red jungle fowl [1]. In Indian poultry farming, ‘backyard poultry system’ is as old as Indian civilization. The exotic and local varieties of the domestic chick, Gallus gallus domesticus, are domesticated in the rural and urban areas where the people use the eggs and the meat as the primary source of protein and also manure as earning source [2]. The disease caused by cestodes is called as cestodiasis.

Gut of domestic fowl is a safe heaven for many cestode parasites, but the tapeworms belonging to the genus Raillietina are the most prevalent avian helminth parasites throughout the world. R. echinobothrida is the most important species in terms of prevalence and pathogenicity, particularly in the domestic fowl, Gallus gallus domesticus [3]. The cestode inhabits the small intestine and causes stunted growth of young chicken, emaciation of the adult, and decreased egg production of the hen [4]. More than 1400 species of tapeworms have been recognized as the cause for cestodiasis in domestic and wild birds throughout the world [5]. The eggs and proglottids of tapeworms should be identified for differentiating one species from the other [6].

In R. echinobothrida infection, the young forms of the parasite penetrate with their anterior end of the body deeply into the mucosa and sub-mucosa of the duodenum of host’s intestine, resulting in the formation of nodules and hyperplasic enteritis at the site of their attachment [7].

In R. tetragona infection, the intestinal wall of the host intestine is thrown into ridges of purplish colour and the intestinal mucosa sloughs off. Raillietinacesticillus is a common tapeworm found in the jejunum of chickens and causes degenerative and inflammatory changes in the intestinal villi. The level of sugar and haemoglobin falls below normal level in the infected birds causing body malfunctions [8].

In the case of Raillietinacechinobothrida (nodular worm), a protuberance or nodule develops in the intestinal wall at the site of attachment of each worm. This is sometimes confused with tuberculosis. Hence, tuberculosis may be diagnosed only in the absence of worm infection [9]. In conditions of heavy infestation R. echinobothrida is listed as one of the most pathogenic tapeworms, causing conspicuous intestinal nodules in chicken, with characteristic hyperplasic enteritis associated with the formation of granuloma [10]. The symptom is termed ‘nodular tapeworm disease’ in poultry. Intestinal nodules often result in degeneration and necrosis of intestinal villi and ultimately lead to death of host. Cestodes being parasites interfere with the physiology of host like they absorb monosaccharides and converting them into glycogen and also absorb amino acids, polypeptides and protein of the host [11].
**Methodology**

For studying histopathological details of tissues, thin paraffin sections were cut with the help of microtome. The material (gut segments) was fixed in Bouin’s fixative for about 24-48 hours and then washed several times in 70% alcohol. The material was then dehydrated through ascending series of alcohol - 50%, 70%, 90%, 100% (I), 100% (II) - for 20-30 minutes in each concentration. Dealcoholisation was achieved with two changes of xylene again for a period of 20-30 minutes. The infiltration of the material for histopathology purposes was done with paraffin wax by keeping it first in a mixture of xylene and wax in the ratio of 1:1 for half an hour at 40-42°C in an oven and finally two changes in pure xylene maintained in a molten state in an oven for 2-4 hours at 50-58°C according to the melting point of wax. Embedding of infiltrated material was done in rectangular paper boats. Fresh embedding paraffin wax from the oven was used to fill the paper boat to the brim. The bottom layer of paraffin wax was allowed to solidify by touching the bottom of container with cold water while the surface layer was kept in melted state with heated forceps. The material was oriented in wax by heated forceps. The boat was kept in cold water and kept in it for 15-60 minutes and then stored in cold place for further processing (Table 1).

The blocks were removed from the paper boats and after trimming them properly, each block was fixed to the block holder. Sections of 5-20 microns were cut with the help of rotator microtome. Continuous ribbons of the material were cut and placed in ribbon carrier. These ribbons were placed in a section tray in the order in which they were cut. A very thin film of affixative was coated on cleaned slides. The affixative used was Mayers albumin. The ribbon of sections was divided into strips of the correct lengths which were then placed on the coated side of the slides in rows. The slides were carefully placed on the hot plate. After removing from the hot plate the slides were kept overnight drying. Removal of paraffin was done by placing slides in pure xylene for about 5 minutes. Xylene was removed from slides by placing them in absolute alcohol for about 5 minutes. The slides were processed through a series of alcohol concentrations in descending order (90%, 70%, 50%, 30%) and then placed in distilled water for 2-3 minutes.

The slides from distilled water were transferred to 0.2% acid fuchsin for 5-10 minutes then to 1% phosphomolybdic acid for 10 minutes to overnight in order to obtain a selective destaining. The slides were next transferred to mallory’s solution 2nd for 50-30 minutes and were then rinsed in phosphotungstic acid. The slides were rapidly dehydrated to 90% from few seconds to 2 minutes in each solution. Dehydration was completed by passing the slides through two changes of absolute alcohol for 3 minutes each. The slides were removed from second change of xylene. A drop of mountant was placed at the left end of each slide. A large cover glass was then placed in contact with the drop of mountant and lowered slowly until it covered the material. The prepared slides were finally cleared by removing the excess mountant with the help of xylene. The slides were then observed under research microscope and photomicrography was done with camera.

**Results and discussion**

In the present work for histopathological studies the tissues from both infected and uninfected hosts were processed for comparison to find out damage done by helminth parasites to the host. The findings are given in photo plates as follows (Figures 1-7):

**Table 1:** Paraffin wax embedding time table.

<table>
<thead>
<tr>
<th>S No</th>
<th>Solution</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bouin’s fixative</td>
<td>Overnight</td>
</tr>
<tr>
<td>2</td>
<td>70% alcohol</td>
<td>2 hours overnight</td>
</tr>
<tr>
<td>3</td>
<td>90% alcohol</td>
<td>20-60 minutes</td>
</tr>
<tr>
<td>4</td>
<td>100% 1st change</td>
<td>20-30 minutes</td>
</tr>
<tr>
<td>5</td>
<td>100% 2nd change</td>
<td>20-30 minutes</td>
</tr>
<tr>
<td>6</td>
<td>100% alcohol xylene in 1:1 ratio</td>
<td>20-30 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Xylene 1st change</td>
<td>20-30 minutes</td>
</tr>
<tr>
<td>8</td>
<td>Xylene 2nd change</td>
<td>20-30 minutes</td>
</tr>
<tr>
<td>9</td>
<td>Xylene saturated with paraffin wax</td>
<td>20-30 minutes</td>
</tr>
<tr>
<td>10</td>
<td>Molten paraffin wax 58ºc 1st</td>
<td>30 minutes - 2 hours</td>
</tr>
<tr>
<td>11</td>
<td>Molten paraffin wax 58ºc 2nd</td>
<td>30 minutes - 2 hours</td>
</tr>
</tbody>
</table>

References:

Davainaproglottina is the most pathogenic tapeworm parasite among all poultry tapeworms. The parasite penetrates deeply within the intestinal villi of host and causes heavy infection leading to necrosis and haemorrhagic enteritis. Its acute forms may be fatal for the host while its chronic infections are characterized by reduced growth rate, emaciation and weakness. Capillary congestion, lymphocytic and eosinophilic infiltrations, fibrosis and proliferation in the gut are also the important microscopic changes associated with tapeworm infection [12]. Tapeworm species under the genera Choaotaenia and Amoebotaenia are not normally pathogenic unless present in very large numbers. However, their infections could cause decrease in production in intensively reared birds [13].

Results and discussion

In the present work for histopathological studies the tissues from both infected and uninfected hosts were processed for comparison to find out damage done by helminth parasites to the host. The findings are given in photo plates as follows (Figures 1-7):
In present work histopathological studies revealed that the intestine of infected fowl showed less growth and more degeneration of villi (Figure 7) in comparison to uninfected intestine which showed normal growth and size of villi (Figure 6). The findings are in line with those of Samad et al. and Pinto et al. [14-15] who have reported atrophied villi, enteritis with cellular infiltration and formation of characteristic granulomas. The gut of infected host showed haemorrhages, lesions, nodular growth, cysts, ulcerations, and eroded mucosal epithelium (Figure 2). The present observations are in conformity with Bhowmik et al. [16]. The haemorrhages, lesions, and nodular growth found in the infective guts in the present study were more or less similar to those found by Dipti et al. and Abdul and Sarkar [17-18]. In general, the degree and extent of enteric changes corresponded to the parasitic load. Attachment of the parasite caused traumatic lesions and erosion of intestinal mucosa which might have favoured secondary bacterial infection and hence more inflammation and severe cellular reaction in the area (Figure 5). The infected intestinal sections were found with embedded scolices (Figure 3). However, delineation of local effects and species specific studies are needed. The higher prevalence of the parasites and observed pathology directly reflects their economic importance and warrants conscious intervention for its control in backyard free range poultry.

Figure 2: Intestinal segment of an infected fowl with embedded scolices, haemorrhages and cysts.

Figure 3: Photo plate showing intestinal segment of an infected fowl with bunch of cestodes with scolices embedded in the intestinal epithelium.

Figure 4: Micrograph showing general histology of gut of an uninfected fowl. The layers shown above are: M-mucosa, SM-submucosa, MC-muscularis, S-serosa.
Figure 5: Micrograph showing a section of infected intestine with a lump of inflammatory cells and mucosal degeneration.

Figure 6: Micrograph showing section of intestine of an uninfected fowl with normal growth and size of intestinal villi.

Figure 7: Micrograph showing section of intestine of an infected fowl with evident degeneration of villi and smaller sized villi called dwarf villi.
The present studies revealed that the infective hosts were weak with down feathers, increased appetite and ruffled plumage, and less weight. These findings were also met by Damae et al. [19] while studying cestodiasis in domestic fowl. Cestodes in poultry are known to cause retarded growth, enteritis, diarrhoea, haemorrhages and hypovitaminosis of vitamin B, heavy infections may also be associated with mortality in young birds and the loss of egg production in egg laying chickens [20].

**Recommendations**

- The relevance of cestodiasis as health problem in domestic fowl will grow if we do not come up with new control measures. Thus, more robust analysis of problem is need of the hour to reveal how these parasites have developed their host/tissue preferences and immune evasion strategies and how this relates to their transmission patterns and distribution. The various recommendations based on current findings and for future studies are mentioned below: Proper disposal of the infected guts to prevent spread of disease by intermediate hosts.

- Farmers raising fowl should improve feed provisions to their animals for good health conditions that confer some level of resistance against cestodiasis.

- Control of cestodiasis either by treatment interventions made by individual farmers or through community involvement is anticipated to increase farm level productivity and household income.

- Curative measures which include the prevention of animal’s access to infection prone areas and biological control of the intermediate host should be well adopted in advance to curb the disease.

- Awareness programmes should be organised to educate the farmers for prevention and treatment methods.

- The housing facilities for chicken should be improved and a feasible number to area ratio should be maintained to decrease unwanted stress on the animal.

- The chicken houses should be cleaned regularly and disinfected to prevent the growth of intermediate hosts.

- Proper treatment of the bird droppings with chemicals to kill any intermediate stage or eggs of the parasite before its use as farm manure.

- Vaccines should be developed for treatment of cestodiasis.

- Lastly the current study recommends an integrated approach to combat cestodiasis which includes broader study of other risk factors like intermediate host habitat and effect of climate. Moreover, variations in the genetics of the cestode population need to be further studied to answer questions on the adaptation and pathological consequences of cestodes in domestic fowl and monitoring of anthelminthic resistance in livestock. Research on identification of stage specific antigens (juvenile, immature and mature stage), their subsequent isolation, recombinant protein expression, biochemical and immunological characterization should be carried out for proper cestode vaccine development.

**References**


