

Morpho-pathological description of first record of fatal concurrent intestinal and renal parasitism in *Columba livia domestica* in India

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ABSTRACT

Out of around 200 pigeons (housed at Mahendra Chaudhury Zoological Park, Chhatbir, Punjab), two pigeons exhibiting signs of depression, lethargy, droopiness and blood tinged greenish diarrhoea were quarantined for detailed examination. The faecal examination revealed a concurrent parasitic egg load of capillarid, ascarid and strongyle eggs. Morphological observations after clearing of retrieved parasites from the deceased pigeons revealed the presence of *Ascaridia columbae*, *Capillaria obsignata*, and *Ornithostrongylus quadriradiatus*. Histopathological studies of the intestines revealed sloughing and hyperplasia of the lining epithelium; whereas kidneys of one of the pigeon exhibited the extensive tubular damage and marked haemorrhages with cut sections of the parasite, *Paratanaisia bragai*. The other vital organs showed no signs of any significant lesions in both pigeons. This seems to be the first report of fatal concurrent gastrointestinal and renal pathology due to *A. columbae*, *C. obsignata*, *O. quadriradiatus* and *P. bragai* in pigeons kept at zoological park in India.

Key words: *Ascaridia columbae*, *Capillaria obsignata*, *Columba livia domestica*, *Ornithostrongylus quadriradiatus*, *Paratanaisia bragai*.

INTRODUCTION

Pigeons, members of the order Columbiformes are distributed throughout the world (Marques *et al.*, 2007). These fascinating birds reside in close vicinity to human dwellings as they have adapted themselves to rural, urban and suburban life styles (Khezerpour and Naem, 2013). Parasitism is an intricate problem in companion birds and animals and is generally overlooked unless it exhibits serious clinical disorders (Tsai *et al.*, 1992). The parasitism affects the birds (especially pigeons) severely, leading to retarded growth, depression, less egg production, immunosuppression and further renders them susceptible to other infections (Dranzoa, 1999). The feeding habits of pigeons include consumption of grains, earthworms, slugs and insects, which make them more susceptible for the invasion of nematode, trematode and cestodes (Adang *et al.*, 2009).

Ascaridia columbae is one of the most pathogenic parasites of pigeons (Kajerova *et al.*, 2004). The target organ of the parasite is mainly small intestines, where it localizes for almost one year. The symptoms in heavily infected birds are diarrhoea, reduction in growth and production, lethargy and depression (Kulusic, 1989). On the other hand, out of the six species of the capillarid nematodes described in the wild and domesticated birds, only two most common species viz. *Capillaria obsignata* and *C. caudinflata* have been incriminated to cause pathogenesis in pigeons (Park and Shin, 2010). Heavy infestations of *Ornithostrongylus*

quadriradiatus has been attributed to exhibit acute symptoms rather than characteristic helminthic chronic disease conditions. Diseased birds lack strength, become lethargic and pass pronounced greenish diarrhoea (Cuvillier, 1937). The trematodes of various genera of the families Eucotylidae and Rencolidae infect the kidneys of the birds and prove detrimental by affecting renal pelvis and proximal ureters (Kanev *et al.*, 2002). *Paratanaisia bragai*, member of the family Eucotylidae had been found involved in infections of kidneys of various bird species, which had proved fatal (Unwin *et al.*, 2013). The prime objectives of present study were morphological identification of the parasites retrieved from the intestines of the pigeons at necropsy and histopathological evaluation of extent of damage caused by them in different organs.

MATERIALS AND METHODS

Faecal sample collection: Amongst the lot of around 200 domestic pigeons, two adults exhibited signs of depression, lethargy, droopiness and blood tinged greenish diarrhoea. Both birds were quarantined for detailed examination. The faecal samples were collected from the infected pigeons as well as from twenty other birds of susceptible population (random sampling) and were kept in polythene bags at 4°C (with the help of ice packs) and then transferred to Department of Veterinary Parasitology, COVS, GADVASU, Ludhiana (India) for further processing. The qualitative analysis was carried out by direct smear method, floatation

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concentration technique using Sheather's sugar solution as well as by sedimentation technique (Soulsby, 1982). Whereas, the quantitative analysis was performed by McMaster egg counting technique to approximate relative parasitic intensity, which was expressed as eggs per gram (EPG) values (Soulsby, 1982).

Gross specimen collection and processing: Despite of immediate therapeutic intervention with albendazole (Albomar®, Virbac India), both pigeons succumbed one after another on consecutive days. At necropsy, markedly dilated intestinal tract stuffed completely with adult parasites and catarrhal exudate was observed. The adult parasites were collected, washed with normal saline and then cleared with lactophenol containing glycerine, lactic acid, phenol and double distilled water (1:1:1:1) for detailed observation and identification. All organs suspected to be affected by the parasitic stages including the intestine, gizzard, liver, lungs, heart and kidneys were collected and fixed in 10% neutral buffered formalin.

Morphological studies: Morphological examination and identification involved morphometry of the eggs as well as dimensional study of the adult parasites. The length and breadth of the eggs (n=20) was depicted in micrometers (μm) and was expressed as mean \pm standard deviation. Similarly, the length of the parasites (n=10 male and female each) was expressed in millimetres (mm) and width in micrometers (μm) and was expressed as mean \pm standard deviation.

Histo-pathological examination: Tissue samples from the intestine, gizzard, liver, lungs, heart and kidneys were collected and processed for histo-pathological studies as per the method of Luna and Lee (1968). Five micron thick sections were cut and stained with routine haematoxylin and eosin stain for the histopathological studies.

Statistical analysis: Data related to morphometric studies was analysed and expressed as mean \pm standard deviation.

RESULTS AND DISCUSSION

Faecal sample examination with direct smear and floatation concentration technique revealed the presence of capillarid, ascarid and strongyle eggs in the faecal samples of both the birds. The quantitative analysis depicted 1200, 2100, 700 and 1600, 1900, 1300 eggs per gram (EPG) values for *Capillaria* and *Ascaridia* species and for Strongyles in the first and second pigeon, respectively. The sedimentation technique carried out for both the faecal samples were observed negative for the presence of any egg. The egg per gram values for other representative pigeons (n=20) of the flock were found to be 710 ± 347.77 , 905 ± 409.72 and 155 ± 131.69 for *Capillaria* species, *Ascaridia* species and for Strongyles, respectively.

The concurrent, heavy gastrointestinal parasitism could be fatal for birds due to the infliction caused by the worms as well as occlusion of the tract (Nghonjuyi *et al.*,

2014). The results of the present study depicted the presence of concurrent gastrointestinal parasitism in domestic pigeons which could have resulted in their mortality. The similar clinical signs observed in the present study were earlier reported by Cuvillier (1937) and Kulisic (1989).

The intestines of both pigeons were completely crowded with roundworms especially ascarids and the findings were in agreement with the results of the earlier studies carried out by Ashrafihelan *et al.* (2010) and Khezerpour and Naem (2013). However, they also reported concurrent presence of cestodes, which was not observed in present study.

The morphological studies targeting the micrometry of the eggs and detailed study of the characteristics features of the adult worms was carried out so as to determine the species of encountered parasites. The average ascarid, capillarid and strongyle eggs (n=10) sizes (length \times breadth) were $80.5 \pm 21.9 \mu\text{m}$ ($79.9 - 94 \mu\text{m}$) \times $44.6 \pm 4.6 \mu\text{m}$ ($37.6 - 51.7 \mu\text{m}$), $51.4 \pm 3.3 \mu\text{m}$ ($47.5 - 56.4 \mu\text{m}$) \times $22.1 \pm 3.2 \mu\text{m}$ ($18.8 - 28.2 \mu\text{m}$) and $72.4 \pm 3.3 \mu\text{m}$ ($70.5 - 79.9 \mu\text{m}$) \times $39.9 \pm 2.5 \mu\text{m}$ ($37.6 - 42.3 \mu\text{m}$), respectively.

The adult ascarid, capillarid and strongyle worms were identified on the basis of gross and microscopic morphological examination. Grossly, the length of the ascarid, capillarid and strongyle male and female worms (n = 5 each) were measured $63.2 \pm 4.7 \text{ mm}$ ($56 - 68 \text{ mm}$) and $70.9 \pm 4.9 \text{ mm}$ ($65 - 76 \text{ mm}$), $10.1 \pm 0.5 \text{ mm}$ ($9.4 - 10.8 \text{ mm}$) and $12.6 \pm 0.2 \text{ mm}$ ($10.8 - 15.0 \text{ mm}$) and $11.8 \pm 1.9 \text{ mm}$ ($9 - 14 \text{ mm}$) and $20 \pm 2.1 \text{ mm}$ ($18 - 23 \text{ mm}$), respectively.

The distinctive morphological features observed in case of ascarid worms were developed triradiate lips (one dorsal and two subventral) with wide cephalic alae extending on both the lateral sides and filariform oesophagus (Fig. 1). Spicules in case of males were almost equal and there was presence of precloacal chitinous-rimmed sucker (Fig. 2).



Fig 1: Anterior end of adult *Ascaridia columbae* with triradiate lips (black arrow), filariform oesophagus (yellow arrow) and wide cephalic alae (white arrow) (4X)



Fig 2: Posterior end of adult male *Ascaridia columbae* with precloacal sucker (white arrow) and spicules (black arrow) (4X)

The male of the *Capillaria* species was possessing oesophagus more than the half of the whole length of the parasite, whereas female possessed shorter oesophagus than males. The vulva was slightly prominent in case of females and was observed slightly posterior to the intestinal - oesophageal union (Fig. 3).

In case of the strongyle worms, the cuticle at the anterior end was inflated forming a vesicular enlargement of about $94.9 \pm 6.1 \mu\text{m}$ (89 -103 μm). The mouth was lacking any visible papillae and was simple and unarmed (Fig. 4). The male parasites were comparatively smaller than the female counterparts. The vulva was situated at an average $4.6 \pm 0.9 \text{ mm}$ (4 -6 mm) from posterior end in female parasites and the body tapered to a blunt, narrow end with a short spine. The eggs were segmented when laid.

The morphological characters of the ascarid worms and eggs, depicting them to be *A. columbae* were in corroboration with the studies of Wehr and Hwang (1964)



Fig 3: Female *Capillaria obsignata* characterized by slightly prominent vulva (white arrow), just posterior to the union of the oesophagus and the intestine (black arrow) (40X)

and Kajerova *et al.* (2004). Whereas, the morphological criteria about the adult worms and the eggs of capillarid parasite in the present study were in agreement with the studies of Wakelin (1965), Soulsby (1982) and Park and Shin (2010), suggesting it to be *C. obsignata*. The characteristics features noticed about the adult strongyle worms and the eggs were consistent with Cuvillier (1937), who reported that *O. quadriradiatus* was pathogenic for domestic pigeons. The morphology of female adult worms was distinguishably clear and could easily be differentiated on the basis of presence of spine at the posterior end. Thus, *A. columbae*, *C. obsignata* and *O. quadriradiatus* were three helminthic species observed in the intestinal tract of both pigeons in the present investigation.

A detailed microscopic examination of the tissues retrieved from the intestines, gizzard, liver, lungs, heart and kidneys was carried out, where marked lesions along with cut section of parasites were observed in intestines and kidneys. Although, no parasites were detected in the heart, lungs, liver and gizzard, but certain microscopic alterations were observed in some organs *viz.* hyperplasia of the lining epithelium with sloughing at places was observed in the gizzard. Most of the lesion in case of intestine was observed in the anterior part of the small intestine *i.e.* duodenum, from where most of the parasites were recovered. At places, cut sections of adult *C. obsignata*, larvae of *A. columbae* and adult of *O. quadriradiatus* with sloughing and hyperplasia of lining epithelium was observed (Fig. 5). Concurrent inflictions to the intestinal tract were in agreement with the findings of Adang *et al.* (2010) and Park and Shin (2010), who observed the adverse effect of *A. galli*, *H. gallinarum* and *C. obsignata* in domestic pigeons and rock partridges, respectively.

The histopathological examination of the kidneys revealed the presence of a digenetic trematode parasite,



Fig 4: Anterior end of *Ornithostrongylus quadriradiatus* with vesicular enlargement (white arrow) (40X)

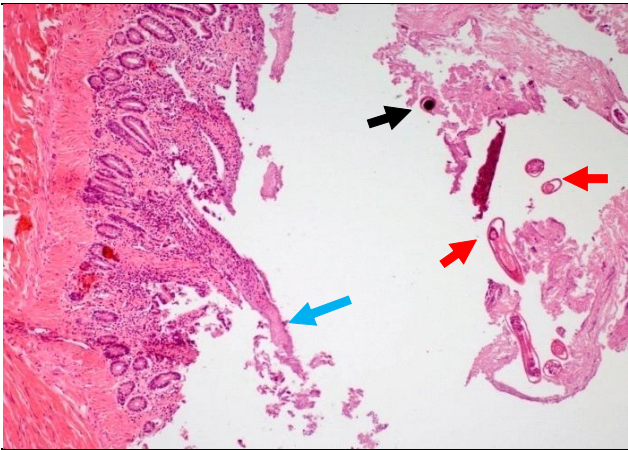


Fig 5: Cut sections of *Capillaria obsignata* (red arrows) and *Ornithostrongylus quadriradiatus* (black arrow) in the lumen of the intestine along with sloughing and hyperplasia of lining epithelium (blue arrow) (10X)

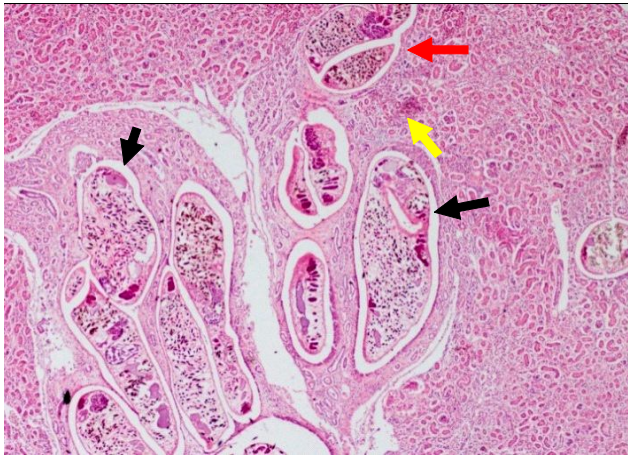


Fig 6: Cut sections of the parasites trapped in renal glomeruli (red arrow), damaging renal tubules (black arrows) and causing marked haemorrhages and infiltration of mononuclear inflammatory cells (yellow arrow) (4X)

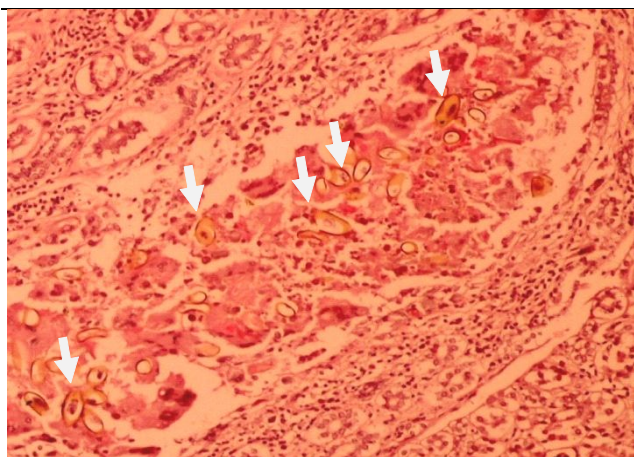


Fig 7: Kidney showing presence of brownish parasitic eggs (white arrows) with tubular damage (10X)

consistent with *Paratanaisia bragai*. The brown coloured eggs, some of which containing fully developed embryos were also observed. Some of the parasites and their eggs were found trapped in the glomeruli, resulting in their destruction (Fig. 6). The major lesions were characterized by haemorrhages and infiltration of mononuclear inflammatory cells around the cut sections of the parasites in the collecting tubules (Fig. 6). The renal tubules exhibited degeneration and presence of brown hemosiderin pigment within the lining epithelial cells. Some of the eggs and hatched miracidium were found free within the parenchyma thereby causing damage to the glomeruli tubules along with infiltration of inflammatory cells (Fig. 7).

P. bragai had previously been reported to parasitize the kidneys of domestic pigeons (Borah *et al.*, 2009), cattle egret (Abdo and Sultan, 2013), guinea fowl (Menezes *et al.*, 2001), whistling ducks (Fedynich *et al.*, 1996), red bird of paradise (Unwin *et al.*, 2013), ruddy ground dove (Pinto *et al.*, 2004), turkey (Brenner *et al.*, 2006) and ring-necked pheasant (Gomes *et al.*, 2005) and the parasite had been incriminated as a cause of death of the heavily infected birds (Arnizaut *et al.*, 1992). The detailed examination revealed that tubular and glomerular damage was primarily due to the parasites as well as by the invading eggs. However, Abdo and Sultan (2013) incriminated the renal damage primarily due to the invading eggs. All other observations in the present study were in corroboration with the findings of Abdo and Sultan (2013), who reported the presence of *P. bragai* in cattle egret. The microscopic changes were also in agreement with the observations of Pinto *et al.* (2004) and Gomes *et al.* (2005).

CONCLUSION

Based on the gross and microscopic findings, authors believe that concurrent intestinal and renal parasitism with the pathogenic parasites resulted in substantial pathology of the organs concerned, which ultimately proved fatal for the hosts. The present study also reflects and supports the fact that concurrent intestinal parasitism leads to depression, diarrhoea, decreased egg production and immunosuppression in domestic pigeons, which further rendered them susceptible to other infections. Based on thorough literature search and available information, this appears to be the first such report of fatal concurrent gastrointestinal and renal parasitism due to *A. columbae*, *C. obsignata*, *O. quadriradiatus* and *P. bragai* in domestic pigeons of Punjab state in India.

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