



Molecular surveillance of *Salmonella* Typhimurium and its respiratory tract pathology in pet birds from Kerala[#]

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Abstract

A total of 76 pet bird carcasses were studied for gross lesions and later histopathology. Molecular investigation detected *Salmonella typhimurium* in 28.6 per cent of psittacine and passerine birds. Twelve out of forty-two and two out of seven psittacine and passerine birds, respectively, were found to be infected with *S. typhimurium*. Regarding columbid and ornamental birds, 22.2 per cent of birds showed positive results. The upper respiratory tract revealed necrotic changes on both the respiratory epithelium and the secretory glands, together with loss of epithelial cilia. Histopathological analysis of respiratory organs of birds positive for the *Salmonella* organisms showed lesions in both upper and lower respiratory tract.

Keywords: Pet birds, *Salmonella*, respiratory tract, histopathology

Pet birds from various orders, such as *Psittaciformes*, *Columbiformes*, *Passeriformes* and *Galliformes* (Ornamental chicken) were chosen for the study. A total of 76 pet bird cases were evaluated for this work. For this investigation, respiratory structures including the larynx, trachea, syrinx, lung and air sacs from 76 pet birds formed the study material. Tissues were collected in 10 per cent neutral buffered formalin and processed to make 5 µm thick sections. The prepared tissue sections were stained with haematoxylin and eosin (H & E) for histopathological examination (Suvana *et al.*, 2018).

The respiratory organs were processed for PCR analysis. The DNA extraction was accomplished using commercial kit (Qiagen). As per the study of Kaabi *et al.* (2019), the following primers were used to amplify the 16S rRNA gene specific for *Salmonella typhimurium*, forward 5'-GGAAGTGAAGACACGGTCCAG-3' and reverse 5'-CCAGGTAAGGTTCTTCGCGT-3'. The PCR cyclic conditions were as follows, initial denaturation at 95°C for 5 min, 94°C for 60 sec, annealing at 65°C for 30 sec, extension at 72°C for 30 sec for 32 cycles, final extension at 72°C for 10 min and held at 4°C. The amplified PCR products were visualised using an agarose gel electrophoresis.

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Among the 76 cases studied, 21 birds were found positive for *Salmonella* typhimurium. The PCR investigation revealed positive results (660 bp) in 28.6 per cent of psittacine and 22.2 per cent of passerine birds. Twelve out of forty-two psittacines and two out of seven passerine birds, respectively, were found to be infected with *S. typhimurium*. Regarding columbid and ornamental birds, 22.2 per cent of birds showed positive results for *S. typhimurium*. The occurrence of salmonellosis in psittacines and passerines identified in this study was lower than that reported by Georgiades and Iordanidis, (2002) who reported 91 per cent in psittacines and canaries and 75 per cent in pigeons. Ibrahim *et al.* (2019) recorded the prevalence rate of *Salmonella* spp. in psittacine birds as 12.9 per cent which was lower than that observed in the current study.

The PCR positive birds showed abdominal distension with ascites (Fig. 1) and sinusitis. The ascitic fluid was serous and straw coloured to pale yellowish. The tracheal mucosa was congested and lungs appeared voluminous due to pulmonary oedema (Fig. 2). Diffuse whitish patches were seen primarily in the medial area of the lungs, while diffuse haemorrhage was more common in the lateral portion. The pulmonary and thoracic air sacs



Fig. 1: Pigeon: Crop dilatation and abdominal distention



Fig. 2: Lung, Grey parrot: Severe pulmonary oedema with diffuse whitish patches and thickening of pleural layer. Pulmonary haemorrhage around the lateral end of the lungs.

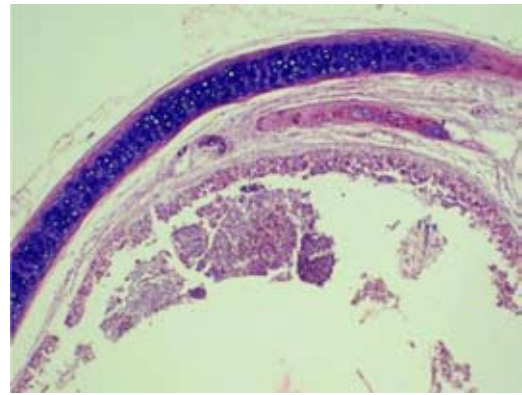


Fig. 3: Larynx, Lorikeet: Necrosis of respiratory epithelium with haemorrhagic contents around luminal epithelium. The mucus secreting cells of secretory glands of submucosal layer were degenerated and infiltrated with lymphocytic cells. H&E, 100X.

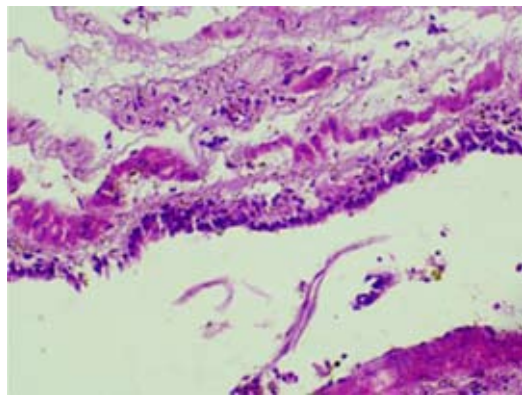


Fig. 4: Bronchi, Lorikeet: Loss of cilia with disorganized lining epithelial cells with focal lymphocytic infiltration. H&E, 400X.

were both thickened and cloudy. The majority of the positive birds had pleuritis with thicker, whitish pleural layers. Histopathological analysis revealed necrotic changes on both the respiratory epithelium and the secretory glands with loss of epithelial cilia. Haemorrhages were present on the luminal surface of the trachea and larynx (Fig. 3). Diffuse and mild infiltration of mixed inflammatory cells, mostly mononuclear cells, in the lamina propria layer (Fig. 4) and distorted ciliary structures (Fig. 5) was observed. A few cases showed localised lymphocytic infiltration in the lamina propria and the layers of respiratory lining epithelium was seen disorganised. Serous to mucus exudates were observed in the parabronchial lumen of the lungs with significant degree of pulmonary oedema. The majority of infected birds had extremely clogged pulmonary vasculature, diffuse haemorrhage surrounding the parabronchi and necrotic debris in the lumen (Fig. 6, 7 and 8). Histological lesions observed in the present study were pulmonary congestion and haemorrhages and similar lesions were reported by Ward *et al.* (2003) in *Salmonella* infected birds. Pulmonary oedema observed in this study in *Salmonella* positive birds was similar to the observation by Vigo *et al.* (2009). Similarly, Dutta *et al.*

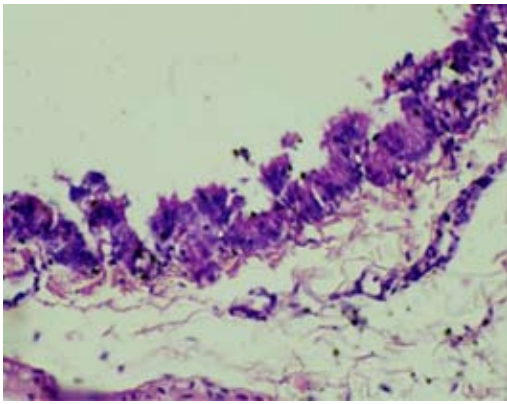


Fig.5: Syringe, Lorikeet: Distortion of cilia structures and fusion of lining epithelium. H&E, 400X.

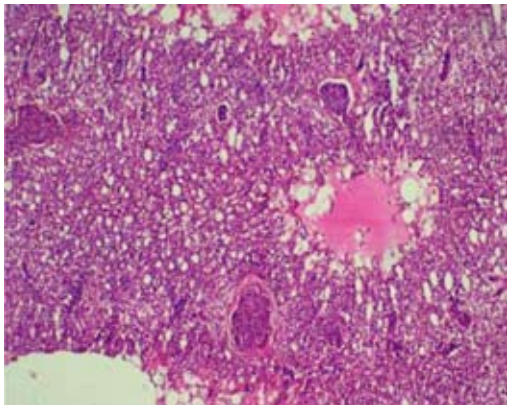


Fig.6: Lung, Budgerigar: Diffuse haemorrhage, severe congestion of pulmonary vessels with serous exudate in parabronchi. H&E, 100X.

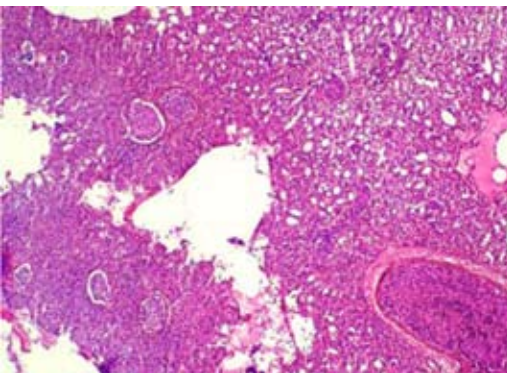


Fig. 7: Lung, Lorikeet: Necrotic debris in the lumen and diffuse haemorrhage H&E. 100X.

(2013), showed that in 12.5 per cent cases, congestion of the lungs was recorded whereas in current study most of the cases had pale lungs with pulmonary oedema. The study of Giovannini *et al.* (2013), reported that interstitial pneumonia was observed in three birds which was contrary to the current study.

Summary

This study showed *Salmonella typhimurium* positivity in 28.6 per cent of psittacine and passerine

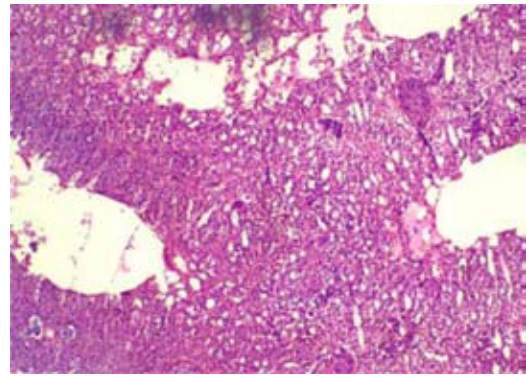


Fig.8: Lung, Lorikeet: Diffuse haemorrhage around parabronchi with oedema. H&E. 100X.

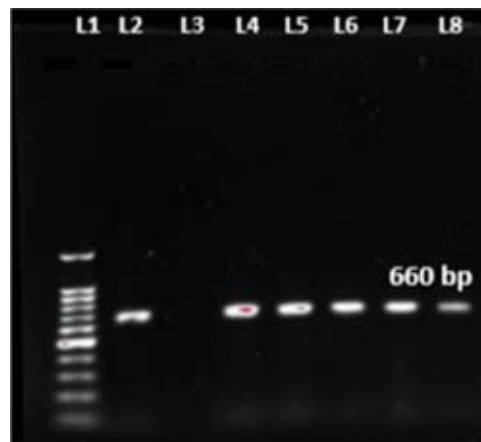


Fig.9: Agarose gel electrophoresis showing amplicons encoding 660 bp of *16srRNA* gene specific to *Salmonella Typhimurium*. The products were visualized by staining the gel with ethidium bromide. Lane 1. 100bp DNA molecular weight marker; Lane 2. Positive control; Lane 3. Negative control; Lane 4 to 8. Positive samples

birds. Twelve out of forty-two psittacine and two out of seven passerine birds, respectively, were found to be infected with *S. typhimurium*. Regarding columbid and ornamental birds, 22.2 per cent of birds were positive for *S. typhimurium*. The upper respiratory tract revealed necrotic changes on both the respiratory epithelium and the secretory glands, together with loss of epithelial cilia. Histopathological analysis of respiratory organs of birds positive for *Salmonella* showed lesions in both upper and lower respiratory tracts. The present report highlights the importance of salmonellosis in causing respiratory infection and mortality in pet birds. Accurate diagnosis, treatment and prevention of such diseases are crucial for economic poultry rearing, including pet birds.

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