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Scientific Report

Avian Polyomavirus Infection in Budgerigars (*Melopsittacus undulatus*) in Turkey*

Özlem ÖZMEN
Department of Pathology, Burdur Faculty of Veterinary Medicine, Akdeniz University, 15100 Burdur - TURKEY
Gerry M. DORRESTEIN
Department of Veterinary Pathology, Faculty of Veterinary Medicine, Utrecht University, Utrecht-THE NETHERLANDS

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Abstract: Twenty-five young budgerigars that died with the complaints of abdominal distension and crop swelling were examined pathologically. One hundred and twelve, 8-19-day-old chicks died at the same aviary with the same clinical symptoms over 16 months; the morbidity and mortality rate was 100%. Ascites, hydropericardium, crop and oesophagus ulcers, cardiomegaly, splenomegaly, hepatomegaly and focal hepatic necroses were the predominant necroscopic findings. The histopathological examination revealed characteristic intranuclear inclusion bodies in the liver, kidney, spleen, lung, oesophagus, crop, brain, pancreas, heart, gut, thymus and bone marrow. Avian polyomavirus infection was diagnosed after an immunohistochemical examination. This is the first report of avian polyomavirus infection in budgerigars in Turkey.

Key Words: Avian polyomavirus, Budgerigar Fledgling Disease (BFD), budgerigar, *Melopsittacus undulatus*, immunohistochemistry.

Türkiye'de Muhabbet Kuşlarında (*Melopsittacus undulatus*) Avian Polyomavirus Enfeksiyonu

Özet: Bu çalışmada, karın ve kursak şişkinliği şikayetleri ile ölen 25 muhabbet kuşu yavrusu patolojik olarak incelendi. On altı aylık bir sürede aynı işletmede 8-19 günlük 112 yavrunun aynı klinik semptomlarla öldüğü, morbidite ve mortalite oranının % 100 olduğu ifade edildi. Asites, hidroperikardiyum, özefagus ve kursak ülserleri, kardiyomegali, splenomegali, hepatomegali ve karaciğerde fokal nekrozlar en önemli nekropsi bulgularıydı. Histopatolojik yoklamada karaciğer, böbrek, dalak, akciğer, özefagus, kursak, beyin, pankreas, kalp, bağırsak, timus ve kemik iliğinde karakteristik intranüklear inkluzyon cisimciklerine rastlandı. Immunohistokimyasal inceleme sonucunda hastalığın avian polyomavirus enfeksiyonu olduğu tespit edildi. Bu, Türkiye'de muhabbet kuşlarında tespit edilen ilk avian polyomavirus enfeksiyonu raporudur.

Anahtar Sözcükler: Avian polyomavirus, Budgerigar Fledgling Disease (BPD), muhabbet kuşu, *Melopsittacus undulatus*, immunohistokimya.

Introduction

Budgerigar fledgling disease (BFD) is a very contagious disease that is caused by the avian polyomavirus (1-3). This virus can cause disease especially in young budgerigars but it can also affect many psittacine and non-psittacine birds (1,3-5). The mortality rate in budgerigar nestlings younger than 15 days of age can be 100% (2,3). The clinical presentation of a polyomavirus infection in budgerigars depends on the age and condition of the bird when exposure to the virus occurs. Neonates from infected flocks may develop normally for 10-15 days and then suddenly die with no premonitory signs. Dysplastic feathers, severe dehydration, ataxia, head tremors, crop stasis, anorexia, paresis and paralysis can be seen (1-3,6). Infections have also been associated with decreased hatchability and embryonic death (1,2). Necropsies reveal various combinations of the following: abdominal distension, petechial to ecchymotic subcutaneous haemorrhages, hydropericardium, ascites, hepatomegaly with focal

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hepatic necrosis, splenomegaly, cardiomegaly, pale or congested swollen kidneys, focal oesophageal mucosal degeneration and abnormalities of feather growth and formation (2,3,6-10).

Feather lesions in surviving budgerigars cannot be macroscopically distinguished from changes caused by the psittacine beak and feather disease virus (circovirus). Malnutrition can also cause feather lesions, which might be difficult to evaluate clinically. Organ lesions can be induced by a variety of infectious agents, particularly bacteria. The demonstration of large pale basophilic or amphophilic intranuclear inclusion bodies is considered suggestive of a polyomavirus infection. A confirmed diagnosis requires immunohistochemical staining of suspected lesions using viral-specific antibodies or the detection of viral nucleic acid using polyomavirus-specific DNA probes (1).

The purpose of the present study was to describe pathological and immunohistochemical findings in a severe infection in an aviary. This is the first report of avian polyomavirus infection in budgerigars in Turkey.

Materials and Methods

One hundred and twelve 8-19-day-old budgerigar chicks in the same aviary died over a 16-month period. No adult birds showed clinical symptoms. Twenty-five of these chicks were brought to the Akdeniz University, Burdur Veterinary Faculty, Department of Pathology for diagnosis. A standard necropsy was performed and tissue specimens were fixed in 10% neutral-buffered formalin for histopathological examination. Formalin fixed tissues were processed routinely, sectioned at 4 µ and stained with haematoxylin and eosin (HE). In some selected cases periodic acid Schiff (PAS) stain was also used after the evaluation of the HE-stained slides. During the necropsy, tissue samples were also taken for microbiological examination.

After the initial histopathological evaluation, tissue samples were sent to Utrecht University, Faculty of Veterinary Medicine, Department of Veterinary Pathology for additional evaluation and immunohistochemical staining. Paraffin blocks were sectioned at 3 µ, attached to glass slides with poly-L-lysine, deparaffinized in multiple xylene baths, and rehydrated in sequentially graded ethyl alcohol baths. Endogenous peroxidase activity was blocked by freshly made 1% hydrogen peroxide in methanol for 10 min. The sections were put in 100%, 96% and 70% alcohol to distilled water and washed in PBS/Tween 3 times. The tissues were incubated with rabbit-anti-avian polyomavirus (APV – Universität Leipzig, Institut für Virologie, Germany) (1:2500 dilution 60 min) and washed again in PBS/Tween. After that they were incubated with swine-anti-rabbit (1:50 dilution 30 min) and washed in PBS/Tween. The sections were incubated with PAP-complex (rabbit) at 1:200 dilution for 30 min and washed with PBS only. After incubation in freshly prepared DAB substrate solution in a dark room, the tissues were washed in distilled water. After counterstaining with haematoxylin and washing in tap water the slides were dehydrated gradually in ethyl alcohol baths and cleared in xylene baths; coverslips were applied with a permanent mounting medium.

Results

The morbidity and mortality rate was 100% in the chicks, which were between 8 and 19 days old. No adult bird suffered from illness or died. The most common clinical findings were abdominal distension (Figure 1), and oesophagus and crop ulcers. Several birds exhibited retarded growth and some had feather abnormalities. Clinical symptoms generally started at 6-8 days of age and birds died 1-2 days after the first clinical symptoms were seen. Death was observed until 19 days of age.

The post-mortem examination revealed ascites, hydropericardium, cardiomegaly, splenomegaly, discoloration and enlargement of the kidneys, hepatomegaly and focal hepatic necroses (Figure 2). Erosions and ulcers were observed in the oesophagus and crops. These organs were haemorrhagic and whitish necrotic material was seen on the mucosa and in the lumen of the crops. Haemorrhages, swelling and necrosis were also seen in the tissues surrounding the oesophagus and crop (Figure 3).

At histopathological examination, pale basophilic intranuclear inclusion bodies were observed in the epithelial cells of the liver, kidney, lung, oesophagus, crop, pancreas and intestine. Inclusion bodies were also seen in the myocardial and endocardial cells of heart, the glial cells of the brain and the mononuclear phagocytic system cells of the spleen, thymus and bone marrow. Fibroblasts, fibrocytes and endothelial cells in the

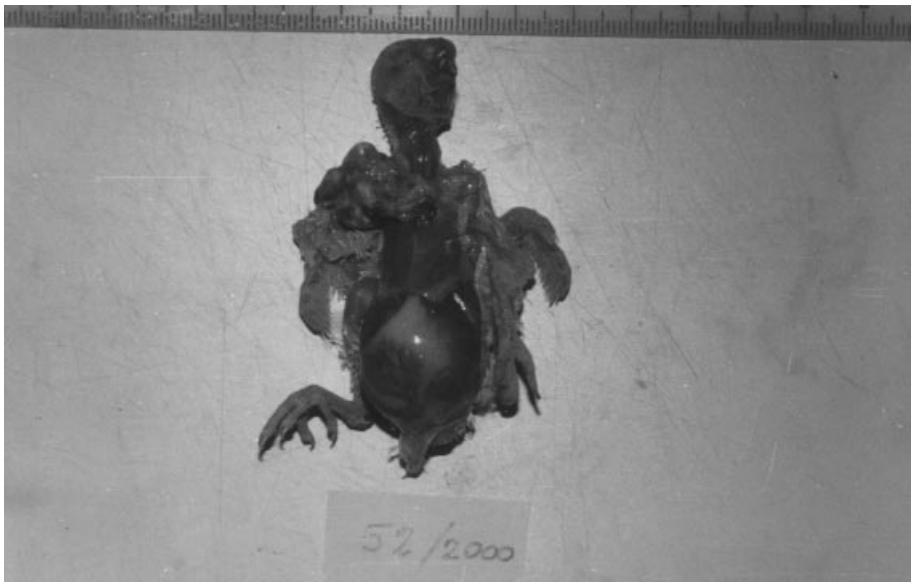

Figure 1. Abdominal distension due to ascites.

Figure 2. Hepatomegaly and cardiomegaly.

mentioned organs were also affected by inclusion bodies (Figures 4-5). Karyomegaly and margination of chromatin with pale basophilic central material were common histopathological findings. Inflammatory reactions, which include predominantly heterophils and macrophages, were seen in the liver, spleen and kidneys. Haemorrhages were also a common finding in visceral organs.

The liver and kidney were most commonly affected; inclusion bodies were observed in all 25 cases in these organs, followed by the spleen (19), heart (17), intestine (10), lung (9), thymus and bone marrow (8), oesophagus and crop mucosa (5), brain (3) and pancreas (1). Focal hepatic necroses were also common findings in the histopathological examination of the liver.

Figure 3. Inflammatory reaction surrounding oesophageal ulcer.

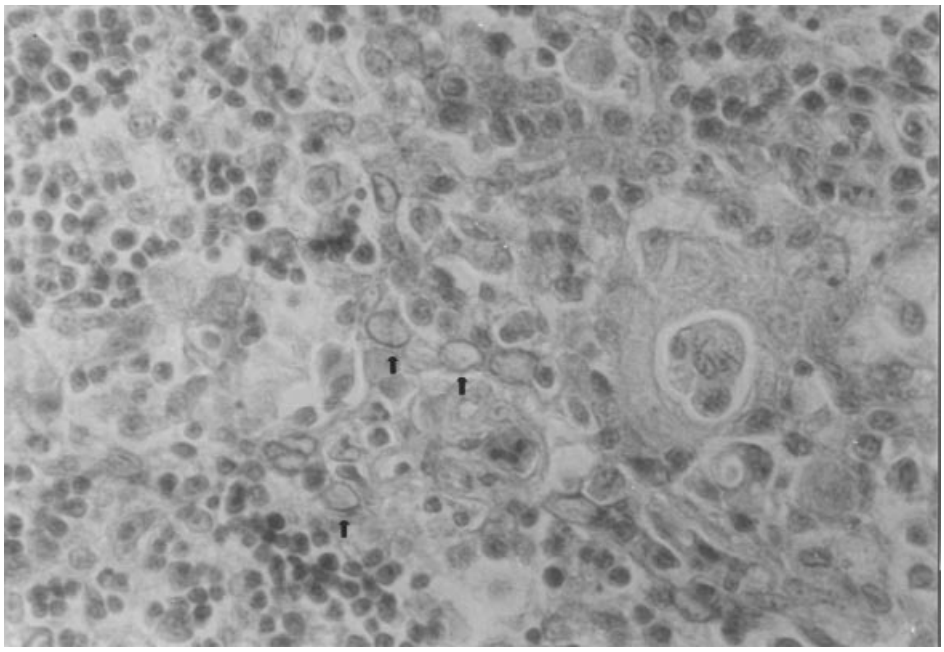

Figure 4. Intranuclear inclusion bodies and margination of the chromatin in the mononuclear phagocytic system cells, thymus (arrows), HE, 400x.

The microscopical examination showed intranuclear pale basophilic inclusion bodies, ulcers and very extensive inflammation in the oesophagus, and crop and their surrounding tissues and muscles. Hyperaemia, oedema, haemorrhages, infiltration of heterophils and macrophages, necrosis and large colonies of bacteria were seen in the inflammation areas. *Escherichia coli* was isolated from organs and especially from crops with

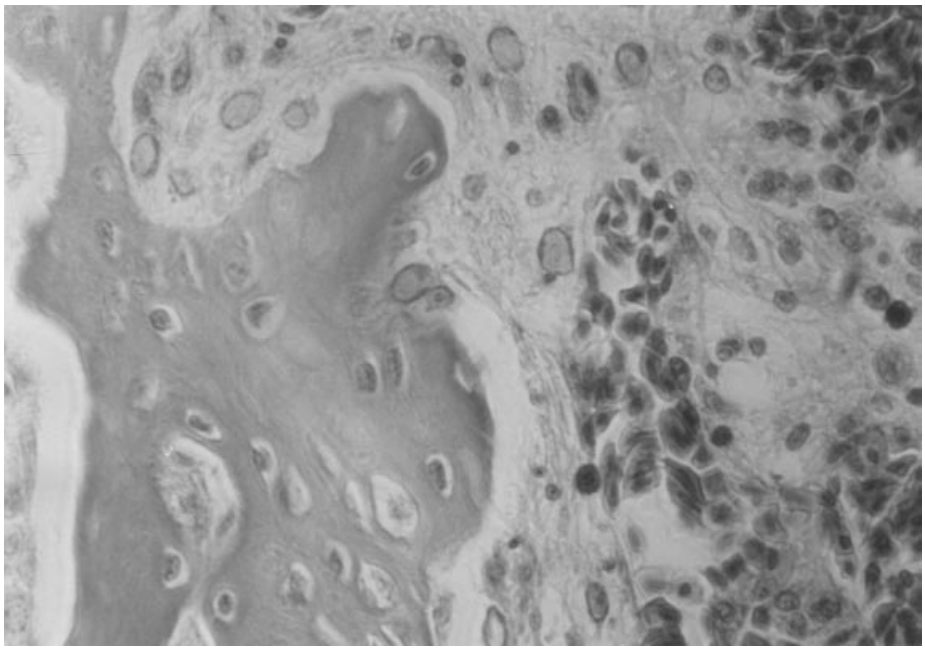

Figure 5. Intranuclear inclusion bodies in the mononuclear phagocytic system cells, bone marrow, HE, 400x.

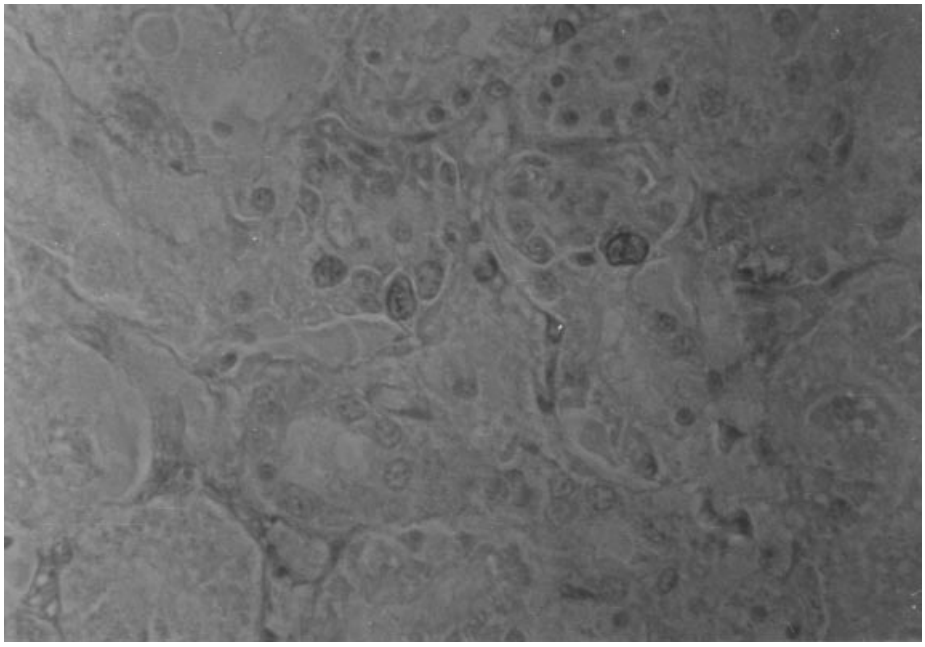

Figure 6. Immunostaining of kidney, avian polyoma virus positive tubular epithelial cells, 400x.

ulcers. PAS staining was negative for mycotic elements in the crops.

The liver, kidney, intestine, heart, bone marrow and thymus were examined immunohistochemically for avian polyomavirus. A strong positive reaction was observed in many nuclei of the epithelial cells in the liver, kidney (Figure 6), intestine and myocardial cells. Fibrous tissue cells, mononuclear inflammatory cells and vessel

endothelial cells also showed a positive reaction in these organs.

Discussion

The avian polyomavirus is one of the most significant viral pathogens of cage birds. It results in substantial economic losses for aviculturists and pet store owners each year (1). BFD can cause multisystemic lesions and death especially in psittacine birds (1,3). Mortality rates vary from 30 to 100% of affected hatchlings (1). In this case the lesions were present in different organs, and the morbidity and mortality rate was 100% in the chicks. The first clinical symptoms (abdominal distension) started at generally 6-8 days of age. After this finding the oesophagus and crop became swollen and haemorrhagic. Birds died 1-2 days after the first clinical symptoms were seen. Growth retardation and feather abnormalities were observed and the birds died after the age of 15 days.

The most frequently affected tissues were the liver and kidney. Mesenchymal cells (fibrocytes, fibroblasts and endothelial cells) predominantly contained viral inclusions but inclusion bodies were also seen in epithelial cells. There was a good correlation between the frequency of viral inclusions in HE staining with the frequency of immunohistochemically positive stained cells. These findings were similar to those of previous studies (11).

In the present study, crop ulcers were observed as a common finding and clusters of bacteria were observed at the histopathological examination of the crops. *E. coli* was the most common secondary bacterial agent isolated from these cases. Some authors have also reported bacterial contaminations in polyomavirus infection (1,12).

Although the histopathological findings are very characteristic for avian polyomavirus, this disease shows a clinical epidemiology very similar to that of psittacine beak and feather disease virus infection (1). The diagnosis must be confirmed by immunohistochemical staining or polymerase chain reaction methods.

Acknowledgements

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