

Pathological, Immunohistochemical, and Electron Microscopic Findings in the Respiratory Tract and Skin of Chickens Naturally Infected with Avipoxvirus

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Abstract: The present study describes pathological, immunohistochemical, and electron microscopic findings in the respiratory tract and skin of chickens naturally infected with avian pox virus. Respiratory distress, depression, and cyanosis were found in the chickens upon clinical examination. At necropsy, erosion, crusts, and nodules were observed on the comb, wattle, and eyelids. Histopathological examination revealed that the epithelial cells in the stratum spinosum were hyperplastic, and that their cytoplasm contained large, eosinophilic inclusion bodies. Epithelial hyperplasia was also prominent in the mucosa of the trachea and bronchi, with characteristic cytoplasmic inclusion bodies and severe submucosal accumulation of mononuclear cells. Avipoxvirus infection was confirmed by an immune positive reaction with antibodies against avipoxvirus within the cytoplasm of the hyperplastic epithelial cells and within the inclusion bodies, and by the electron microscopy of typical dumbbell-shaped poxviral particles within the epithelial cell inclusions.

Key Words: Chicken pox, pathology, immunohistochemistry, electron microscopy, skin, respiratory tract

Kanatlı Çiçek Virüsü ile Doğal Enfekte Tavukların Deri ve Solunum Kanalında Patolojik, İmmunohistokimyasal ve Elektron Mikroskopik Bulgular

Özet: Bu çalışmada, kanatlı çiçek virüsü ile doğal enfekte tavukların deri ve solunum kanalında patolojik, immunohistokimyasal ve elektron mikroskopik bulguların bildirilmesi amaçlandı. Klinik olarak, tavuklarda solunum güçlüğü, depresyon ve siyanoz dikkati çekti. Nekropside, ibik, sakal ve göz kapaklarında erozyon, kabuk ve nodüllere rastlandı. Histopatolojik olarak, stratum spinosumda epitel hücrelerinde hiperplazi ve sitoplazmalarında iri eozinofilik inklüzyon cisimcikleri görüldü. Benzer biçimde epitelial hiperplazi, sitoplazmik inklüzyon cisimcikleri ve submukozal mononükleer hücre infiltrasyonuna trakea ve bronş mukozasında da rastlandı. Avipoxvirus enfeksiyonu, hiperplastik epitel hücrelerinin sitoplazması ve inklüzyon cisimciklerinin antikörlerle pozitif immun reaksiyonu ve elektron mikroskopta epitelial inklüzyon cisimciklerinde çan benzeri tipik poxvirus partiküllerin varlığı ile tespit edildi.

Anahtar Sözcükler: Tavuk çiçeği, patoloji, immunohistokimya, elektron mikroskopi, deri, solunum kanalı

Introduction

Avian pox is an infectious and contagious viral disease of numerous domestic and wild birds, which occurs particularly in tropical and sub-tropical countries (1,2). Outbreaks of the disease still affect the poultry industry in many countries, with infection occurring through the

mechanical transmission of the virus to injured skin or through transmission by mosquitoes and mites (3,4). Despite the variety of hosts, pox virus infection is manifested either cutaneously or diptheritically, and both forms of the disease may occur in the same bird (5). In the cutaneous form, the proliferative lesions are primarily

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confined to featherless areas of skin, while in the more lethal diphtheritic form, the lesions may be found in the upper digestive and respiratory tracts (1,3,5-11). In the mild cutaneous form of pox disease, flock mortality is usually low, but it may be high when the infection is of a generalized diphtheritic form or when the flock is affected by a secondary infection, usually in poor environmental conditions (10). The present study describes pathological, immunohistochemical, and electron microscopic findings in the respiratory tract and skin of laying hens naturally infected with pox virus.

Material and Methods

Chickens

Twelve (8 alive and 4 dead) chickens, of various ages and about 1.5-2.5 kg in body weight, from a flock of approximately 100 white leghorn laying hens housed in small cages next to a gasoline station, were brought for diagnosis to the Department of Veterinary Pathology, University of Kafkas, Kars, in March, 2003. Some of the chickens in the flock showed crusts or nodules on the comb, wattle and eyelids. Moreover, it was reported that several chickens had died at irregular intervals until the end of March, 2003. Necropsy was conducted on all the chickens submitted for diagnosis.

Histopathology

Tissue samples were taken from the comb, wattle, eyelids, lungs, trachea, heart, liver, spleen, kidneys, cerebrum, and cerebellum and fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E). Some sections from the skin and the lungs were also stained with periodic acid-Schiff (PAS).

Immunohistochemistry

The tissue specimens were stained using an indirect immunoperoxidase technique (12), employing a polyclonal chicken anti-pox virus hyperimmune serum. All of the sections were dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase was blocked with 3% hydrogen peroxide. After being washed with phosphate-buffered saline (PBS) for 5 min, the slides were incubated with normal rabbit serum for 30 min at ambient temperature. The sections were incubated with a polyclonal chicken anti-avipoxvirus hyperimmune serum at a concentration of 1/64 in PBS for 30 min.

Finally, the sections were incubated with horseradish peroxidase (HRP) conjugated with rabbit anti-chicken IgG at a concentration of 1/200 in PBS for 30 min. The tissues were washed in PBS after each step. Immunostaining was obtained using 3,3 diaminobenzidine as the chromogen. Hematoxylin was used as the counterstain. The primary antibodies were omitted and replaced by PBS for negative controls.

Transmission electron microscopy

A pox lesion was identified by light microscopy, and the adjacent tissue was excised from the wax embedded specimen. The sample was dewaxed, post-fixed with osmium tetroxide, and embedded in epoxy resin; then thin sections were prepared and examined in a Philips 201 transmission electron microscope (TEM).

Results

Gross findings

Clinically, the live chickens showed conjunctivitis, depression, anorexia, a decrease in egg production, and cyanosis on the skin. The most prominent findings detected in all of the chickens were hemorrhagic erosions, crusts or nodules on the comb, wattle, and eyelids (Figure 1).

Histopathology

Histopathologic examination of lesions in all necropsied chickens revealed similar pathology, mainly characterized by the presence of epidermal hypertrophy and hyperplasia in cutaneous lesions, with ballooning degeneration of stratified squamous epithelium. Epithelial cells were commonly swollen, rounded, and separated from each other in the stratum spinosum layer. Cytoplasm of the hyperplastic epithelial cells contained characteristic large eosinophilic inclusions (identified as Bollinger bodies) and vacuoles (Figure 2). Inclusions distended the cell cytoplasm, producing cell necrosis. In some chickens, moderate heterophilic infiltrations in the dermis could also be observed. The superficial epidermis of the lesions was ulcerated with eosinophilic, amorphous keratinaceous crusts, necrosis, and numerous degenerated granulocytes. Diffuse bacterial colonies were detected in lesions in which there was ulceration of the keratinized superficial epidermis.

In addition to skin lesions, severe epithelial hyperplasia was found in the mucosa of the trachea and bronchi (Figure 3) of 3 chickens, causing partial



Figure 1. Nodules associated with erosions and crusts on the comb and eyelid of a chicken.

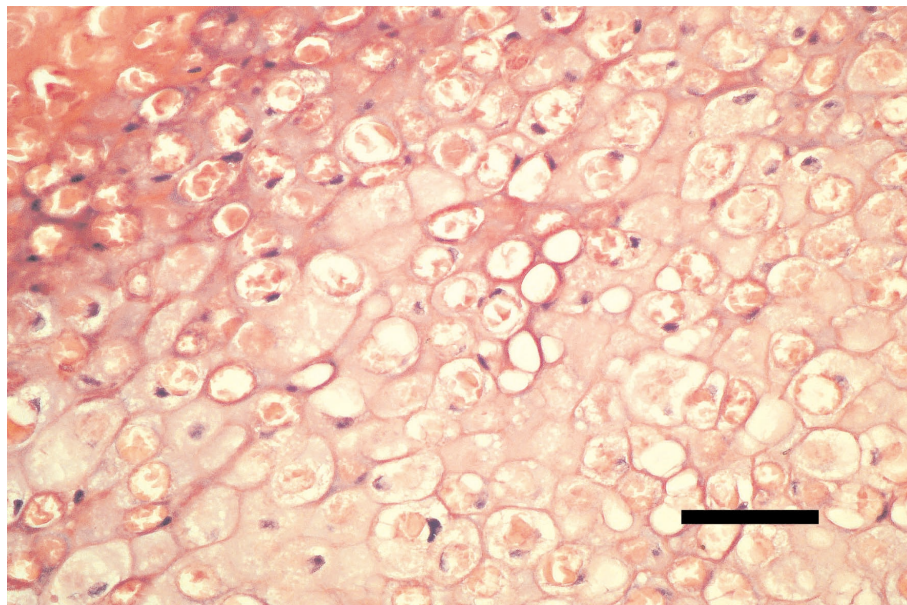


Figure 2. Numerous eosinophilic intracytoplasmic inclusion bodies of various sizes in the hyperplastic epithelium of the comb. H&E, Bar = 51 μ m.

obstruction of the airways, and numerous intracytoplasmic pale-eosinophilic inclusion bodies were present within the hyperplastic cells. Within the underlying lamina propria and muscularis, there was severe accumulation of mononuclear cells, composed mainly of macrophages, lymphocytes, plasma cells, and a

few heterophils. Numerous Russell bodies, globoid and of varying size, were found both in the cytoplasm of plasma cells and free around the mononuclear cell accumulation. The bronchial lumina were occluded by necrotic and desquamated epithelial cells, heterophils, erythrocytes, colonies of bacteria, and amorphous eosinophilic material.

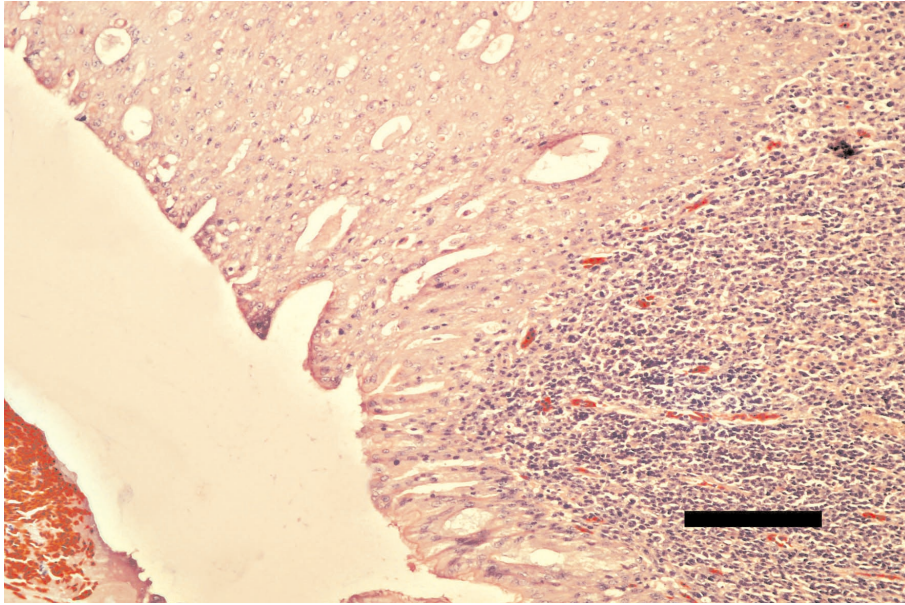


Figure 3. Severe epithelial hyperplasia with accumulation of mononuclear cells in the lamina propria of the bronchi. H&E, Bar = 166 μ m.

In the lung parenchyma, focal lymphocellular accumulation and macrophages with cytoplasmic carbon particles were found. The cytoplasm of the epithelia in both skin and lungs was unstained with PAS. Marked lymphocellular depletion and haemosiderosis were clearly observed in the spleen. A few lymphoid foci, perivascular heterophil accumulation, and diffuse lipid vacuoles were found in the liver. In the intestines, worm infestation (probably *Amoebotaenia cuneata*) and heterophil infiltration were seen in 2 chickens.

Immunohistochemistry

Immunohistochemically, in both the skin and respiratory duct, hyperplastic epithelial cells reacted strongly with antibodies against avipoxvirus. The immunoreaction was granular and occurred mainly in the cytoplasm of the infected cells, in the inclusion bodies, and in the necrotic and desquamated cells in the stratum corneum of the skin (Figure 4). Viral antigen was also detected in the cytoplasm of a few macrophages in the dermis.

Transmission electron microscopy

Virus particles with typical avipoxvirus morphology were identified by TEM. Inclusion bodies were seen to consist of numerous, dumbbell-shaped bodies typical of pox virions (Figures 5 and 6). Viral particles were also

observed to be closely adherent to the host cell membrane in areas of microvillus effacement.

Discussion

Although the problems caused by the disease have been reduced in poultry production, avian pox remains a significant pathogen, and field cases of the disease are observed frequently in chickens (3,9) and in many wild bird species (4,8,10,11,13). The literature review showed that the disease in most birds is mild and rarely results in deaths, particularly with cutaneous pox. However, when pox virus infection spreads to the mucous membranes of the oral cavity and upper respiratory tract, or when the flock is affected with a secondary infection mostly in poor environmental conditions, mortality rates are usually higher (2,10,13). Similarly, our study found relatively high mortality in the present cases. Although it was not possible to perform a bacterial culture in the chickens, the high level of mortality in the flock might have been due to the secondary infection, decreased resistance in the birds, the unusual pathogenicity of the disease agent, or the housing of the animals in unsuitable cages next to a gasoline station, as stated in previous studies (2,4,9).

The cutaneous lesions found on the comb, wattle, and eyelids in the present study were the predominant

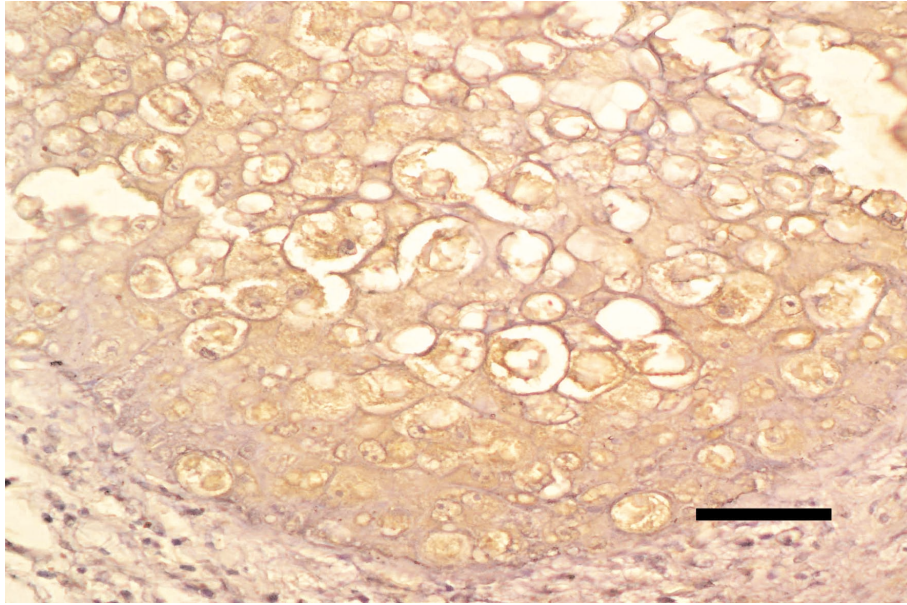


Figure 4. Positive immunoreaction of hyperplastic epithelial cells with inclusion bodies for avipoxvirus antibodies in the comb. ABPC, Bar = 51 μ m.

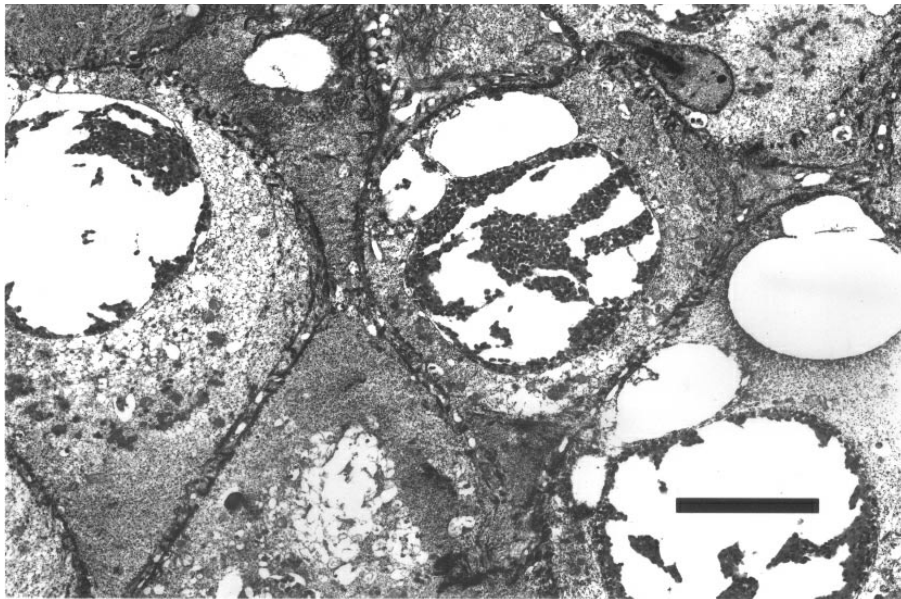


Figure 5. Comb tissue: epithelial cells with pox viral inclusions in the epidermis. TEM, Bar = 5 μ m.

pathological features of the disease in all necropsied animals, as described in previous research (5,7,10,14-17). It has been reported commonly that avipoxvirus-induced changes are mainly characterized by ballooning degeneration and proliferation of epithelial cells, and the formation of cytoplasmic inclusion bodies (1,5,8,10,14,16,18-21), as observed in all cases in this

study. The clusters of epithelial cells found in this study resembled a papilloma, probably formed by basal cell proliferation in the epidermis, as described in previous studies (10,11,19). The hyperplastic lesions caused by avipoxvirus are categorized pathologically between tumor and inflammation (16,17,19).

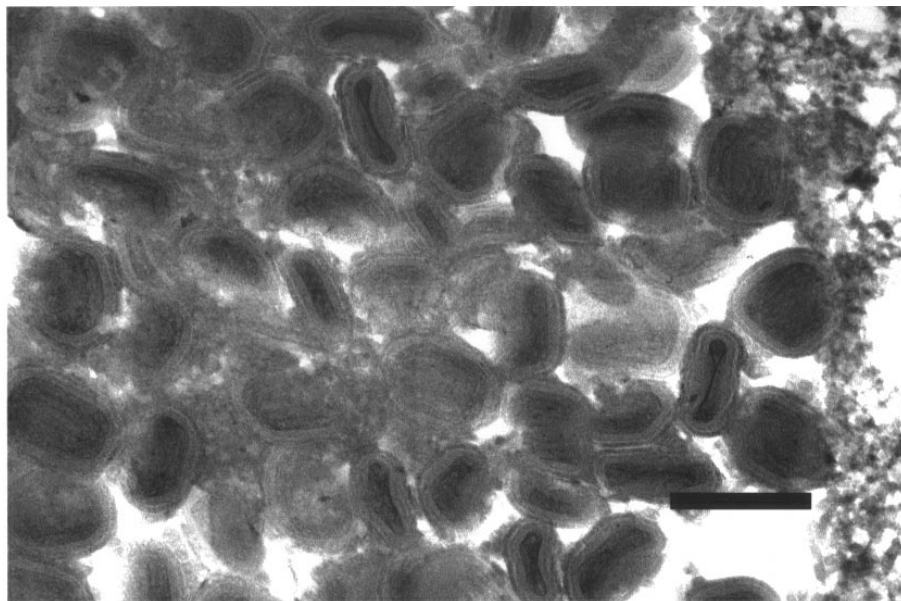


Figure 6. Comb tissue: typical dumbbell-shaped poxviral particles within the epithelial cell inclusions. TEM, Bar = 500 nm.

In the cases studied here, it was noted that hyperplastic epithelial cells containing cytoplasmic inclusion bodies were separated from each other, and were larger and rounder than the epidermal cells without inclusion bodies. Tanizaki et al. (19) reported that as inclusion bodies increased in size the epithelial cells distended, resulting in degeneration and necrosis of the cells. They further suggested that the separation of virions from the tracheal epithelial cells was probably the result of desquamation and necrosis of the cells. Thus, it may be said that the epithelial cell changes found in these cases, including the loss of orientation and organization, might be the result of viral propagation in the cells, compatible with the results reported by Tanizaki et al. (19).

In the mucosal form of the disease, avipoxvirus targets the mucosa of the beak cavity, pharynx, larynx, and, rarely, the lower respiratory epithelia (2,8,9,11). Singh et al. (22) reported that multiplication of avipoxvirus in the lungs follows a cutaneous infection, and implicates a passage of the virus to the lungs through the lymphatics or the blood stream. In this study, the lower respiratory epithelia were markedly affected and were characterized by severe hyperplasia of epithelial cells in the trachea and bronchi, an accumulation of mononuclear cells in the underlying layers, and the formation of Russell

bodies in the lung parenchyma next to the bronchi, which confirms a chronic infection, consistent with observations in hens (18) and lories (8). Although *Avipoxvirus* is known to induce mild to moderate immunosuppression, the lesions found in other parenchymal organs were not specific for the disease, and might have been induced by an additional etiology, as reported previously by Gerlach et al. (8).

The present study found that immunostaining of viral antigen, in both the skin and respiratory tract, occurred mainly within the cytoplasm of hyperplastic epithelial cells with cytoplasmic inclusion bodies, consistent with previous observations in quails (21). These results strongly confirm that viral replication occurs in the cytoplasm of the virus-infected epithelial cells and the immature virions penetrate the inclusion body vacuoles, where they mature (3,20). It should also be stated that immunoreactivity was more intense in the epithelial cells of the skin with cutaneous lesions than in the respiratory tract, and this is compatible with the formation of numerous and large inclusion bodies in the skin.

Transmission electron microscopy found numerous typical dumbbell-shaped poxvirus particles both within the cytoplasm of hyperplastic epithelial cells and in the inclusion bodies, consistent with other studies on various birds (10,11,21).

In conclusion, histopathological findings, along with gross cutaneous lesions, indicated a poxvirus infection in the white leghorn chickens. However, the presence of the typical skin lesions is not sufficient to definitively diagnose avian poxvirus infection because nutritional deficiencies, mycotoxins, and other agents, such as papilloma virus or scaly leg mites, could produce similar lesions (11). Consequently, even though epithelial hyperplasia and Bollinger bodies are histologically indicative of an avian poxvirus infection,

immunohistochemistry and electron microscopy should be applied to ensure a reliable diagnosis of the disease.

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