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Research Article

Avipoxvirus Infection in Quails

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Abstract: The present study describes clinical, virological, histopathological, immunohistochemical and electron microscopic findings of pox infection encountered in a quail flock. Lesions consisted of single or multiple nodules with a crust in variable size of gray to yellow or dark brown discoloration on the comb, eyelids, and the other poorly feathered areas of the body. Histopathological changes consisted of hyperplastic epithelium overlying, with ballooning degeneration of keratinocytes, many of which had eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies), and heterophilic infiltrations admixed with mononuclear cells extending into dermis. Avipoxvirus infection was confirmed by positive immunostaining in the cytoplasm of affected cells for poxvirus antigen, excluded no immunostaining of inclusion bodies, using streptavidin-biotin peroxidase complex method and by demonstration of brick-shaped virus particles with a central core using direct electron microscopy. Typical thickness of chorio-allantoic membranes (CAMs) infected with virus, compared with controls, was observed, and the harvested virus on CAMs was detected as positive against known fowlpox virus using gel diffusion test.

Key Words: Avipoxvirus, pox, quail

Bıldırcınlarda Avipoxvirus Enfeksiyonu

Özet: Bu çalışmada, bir bıldırcın çiftliğinde karşılaşılan çiçek olgusunun klinik, virolojik, histopatolojik, immunohistokimyasal ve elektron mikroskopik bulguları incelendi. Lezyonlar ibik, göz kapakları, ve vücudun kılsız bölgelerinde değişik büyüklüklerde, gri-sarı veya koyu kahverengi kabuklu, tek ya da çok sayıda nodüllerden ibaretti. Histopatolojik olarak, derideki hiperplastik epitelde keratinositlerde balonumsu dejenerasyon ve bu hücrelerde eozinofilik intrasitoplazmik inklüzyon cisimcikleri (Bollinger cisimcikleri) belirlendi. Ayrıca dermise kadar uzanan mononüklear hücrelerle karışık halde heterofilik hücre infiltrasyonları gözlendi. Avipoxvirus enfeksiyonu, streptavidin-biotin peroksidaz kompleks metot kullanılarak yapılan boyanmalarda poxvirus antijeni etkilenen hücrelerin sitoplazmasında saptandı. Elektron mikroskobik incelemede ise, merkezi korlu, tuğla şeklindeki avipoxvirus partikülleri görüldü. Virusla enfekte korio-allantoik membranlar (CAM) kontrollerle karşılaştırıldığında tipik olarak kalınlaşmıştı ve CAM'da üretilen virus, gel diffüzyon testi ile bilinen fowlpox virusa karşı pozitif olarak belirlendi.

Anahtar Sözcükler: Avipoxvirus, çiçek, bıldırcın

Introduction

Avian pox is an acute contagious disease caused by genus Avipoxvirus, including fowl, turkey, pigeon, canary, junco, quail, sparrow, and starling poxviruses in the family Poxviridae, and has been reported in a wide variety of domestic and wild birds (1-3). The host antigen-related viruses known to affect avian species are in general species-specific, but may infect host of other avian species (3,4). It is a slow-spreading disease characterized by development of discrete nodular proliferative skin lesions on poorly feathered parts of the body (cutaneous form) or fibrino-necrotic and proliferative lesions in mucous membrane of upper respiratory tract, mouth, and esophagus (diphtheritic form). Significant mortality has been observed in quails, especially with the diphtheritic form involving the respiratory tract (3).

Avipoxvirus infection is rare in quails; however, the presence of the disease has been reported in various quail species in some countries (5-8). Most of those studies are of wild birds in America. However, there has been no detailed information about immunohistochemical detection of viral antigen in naturally occurring poxvirus infection in Japanese quails. This paper describes clinical, virological, histopathological, immunohistochemical and electron microscopic findings of naturally occurring poxvirus infection in a quail flock in Van province, Turkey.

Materials and Methods

Birds. In the year 2000, twenty sick (no: 8) or dead (no: 12) quails (*Coturnix coturnix japonica*) were obtained from an outbreak of pox infection encountered in an egg-laying quail flock in Van province, Turkey. Approximately 3000 birds of different ages were in the flock and the infection was not encountered in young quails or chicks separated from other sick adult birds. Necropsy was performed on the birds euthanized under ether anesthesia or immediately following death. Tissue specimens were collected from various organs, then fixed in 10% neutral-buffered formalin solution. The tissues were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E). Additional fresh tissues from scabs were collected for virological studies.

Chicken embryo inoculation. Scabs collected from affected quails were triturated to make a 10 % suspension in phosphate buffer saline (PBS). After centrifugation for 30 minutes at 2000 g, the supernatant was inoculated at 0.2 ml doses on chorio-allantoic membranes (CAMs) of 10-day-old chicken embryos. The infected CAMs were harvested 7 days after inoculation.

Gel diffusion test. The harvested virus on CAMs was suspended in PBS, and then the mixture was centrifuged for 20 minutes at 1000 g. The supernatant was tested for precipitating potency against known positive fowlpox virus antisera. The test was performed in plastic petri dish containing 0.7 percent agar noble and examined for line of precipitation after 48 hours.

Immunohistochemistry. Investigation for the distribution of avian poxvirus antigen using streptavidin-biotin complex method (ABC) was performed with a commercial kit (Shandon Inc., Pittsburgh, PA, USA). The technique included an enzyme (protease) pretreatment and all deparaffinized sections were quenched for endogenous peroxidase with 3% H_2O_2 in PBS and blocked with 5% normal goat serum prior to exposure to primary antisera. The sections then were incubated with primary rabbit polyclonal antibodies raised against fowlpox virus (Poultry Diseases Research and Vaccine Production Institute, Manisa, Turkey), diluted 1:200 in PBS for 4 hours at room temperature. The antisera were prepared as previously described (9). The sections were incubated with the biotinylated secondary antibody, followed by streptavidin-peroxidase conjugate, and then visualized with 3-amino-9-ethyl carbazole and counterstained with Mayer's hematoxylin. The primary antibodies were omitted and replaced by PBS for negative controls. Tissue sections known to express the poxvirus antigens served as positive controls.

Electron microscopy. Preparation was carried out using the flotation technique. First, fresh tissue samples as per the scabs for inoculation on CAM were homogenized to a 40% suspension in distilled water. Each mixture was centrifuged for 20 minutes at 1000 g. A carbon-coated electron microscope grid was floated on a drop of the supernatant for 45 minutes, removed and blotted with filter paper, then negatively stained with 1% phosphotungstic acid and dried. The prepared grids were examined in a transmission electron microscope (Jeol 100 CX-II).

Results

Clinically, sick birds showed weight loss, decreased egg production and impaired fertility. Mortality and morbidity in adult birds of the quail flock were approximately 20% and 60% respectively. The disease was characterized by the cutaneous form comprising small multifocal to coalescing nodular lesions in variable size of gray to yellow or dark brown discoloration on predominantly affecting the comb, eyelids, and the other poorly feathered areas of the body. When the brown wart-like scabs coating the extensive thick lesions were removed, a variable amount of caseous exudates admixed with blood was found. Many of affected birds had unilateral or bilateral blindness due to mild to severe blepharitis and conjunctivitis (Figure 1). In such birds, opacity of the cornea due to keratitis was a common finding. Some birds had brown crusty papules at the commissure of the beak and around the external nares. In three cases necropsied, superficial small raised white-yellow nodular lesions at hard palate and the base of tongue were also observed, but involvement of other visceral organs was not seen in any birds.

Microscopically, the affected skin, feather follicles and conjunctiva showed varying degrees of epithelial hyperplasia, covered by a necrotic material, with ballooning degeneration of keratinocytes with vacuolated cytoplasm and intraepithelial vesicles. Numerous degenerated and enlarged keratinocytes, especially in the epithelium of skin and feather follicles, contained eosinophilic cytoplasmic inclusions (Bollinger bodies) with central pale zone (Figure 2). In the dermis and subconjunctiva, edema and congestion with necrosis and

Figure 1. Typical pox lesions characterized by red-brown wart-like masses on snood and comb of a quail. Note obstruction of vision caused by a complicated eye lesion with caseous material.

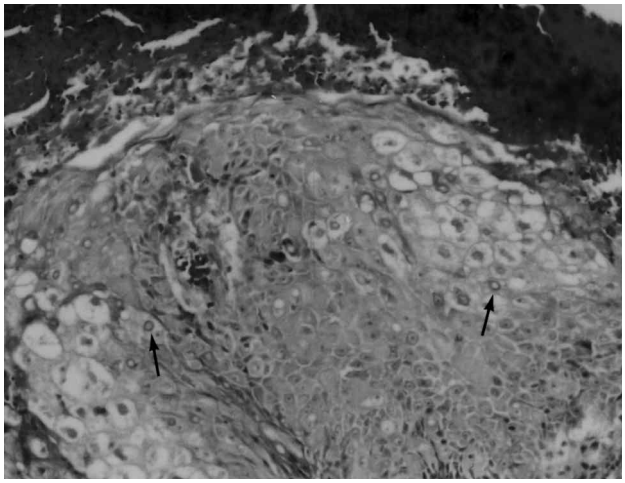

Figure 2. Hyperplastic and degenerative changes in the epidermal layer covered by necrotic material. Arrows indicate intracytoplasmic inclusion bodies. H&E, 200X.

predominantly heterophilic inflammatory cell infiltrations admixed with mononuclear cells were observed. In the birds with corneal opacity, the anterior chamber and limbus cornea was infiltrated with heterophil leucocytes. Some of the birds had focal mononuclear cell infiltrations in the lungs, spleen, liver and proventriculus.

Immunolocalization of poxvirus antigen seen as a diffuse or granular brick-red coloring of the cytoplasm almost confined to the epithelium of skin and conjunctiva (Figures 3, 4). Positive immunostaining were observed in the cytoplasm of degenerated or exfoliated cells in the epidermis, feather follicle and conjunctiva. Viral antigen was identified occasionally in the cytoplasm of macrophages in the dermis and in the subconjunctival propria (Figure 4). Corneal epithelium showing opacity did not include positive immunostaining. Some acinar cells in the lacrimal glands had positive immunostaining. The inclusion bodies present in the cytoplasm of the epithelial cells did not stain by ABC method (Figure 5). Viral antigen was not detected in other organs examined.

Virus particles with a typical avipoxvirus morphology using a transmission electron microscope were identified. Each virion consisted of a brick-shaped with a centrally placed core. The outer coat was composed of randomly distributed surface tubules (Figure 6).

The inoculated CAMs were thicker than the controls, but neither histopathological nor immunohistochemical examination was performed. The harvested virus on CAMs was detected as positive against known fowlpox virus using gel diffusion test.

Discussion

The clinical signs and histologic lesions observed in the affected quails were similar to those in the previously described cases of natural avipoxvirus infection in quails (5-8,10) and other avian species (11-14). Lesion morphology and distribution were typical of avian pox, with a high prevalence of lesions involving the skin of the head, presumably the poorly feathered areas. In the diphtheric form, superficial small raised white-yellow nodular lesions limited on the hard palate and the base of tongue with low percentage of cases and the other visceral organ involvement was not observed. The atypical pox lesions are reported in the feathered parts of the body in chicken, mainly in the posterior dorsal area and external part of the thigh (15,16). In the present study, no lesion observed in other cutaneous areas, or in the viscera besides three birds with superficial small raised white-yellow nodular lesions at hard palate and the base of tongue. Ocular lesion with corneal opacity and conjunctivitis has been reported in the previously reported avianpox infections (7,17). In the present study, corneal opacity was attributed the presence of the inflammation in the anterior chamber and corneal limbus, which extended from conjunctival epithelium. The emaciated condition of the birds, which is common in

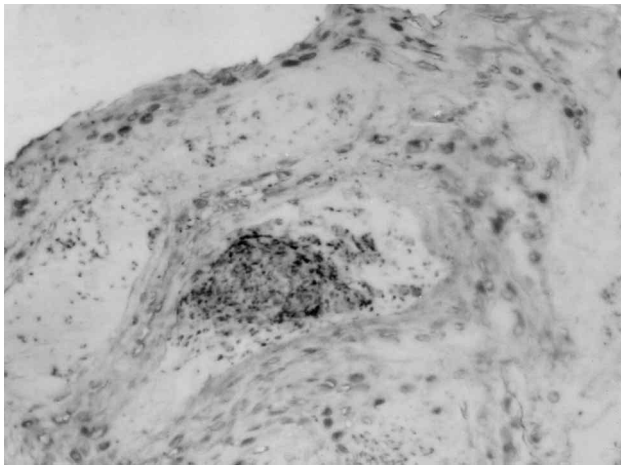

Figure 3. Positive immunostaining for avian poxvirus antigen in the epidermis. ABC method, Mayer's hematoxylin counterstain, 80X.

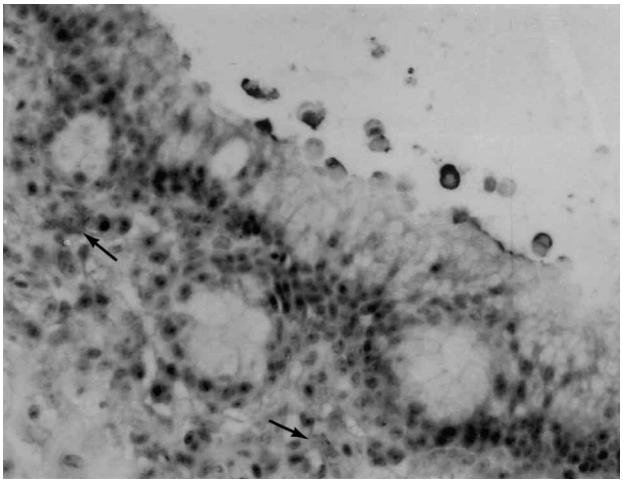

Figure 4. Positive immunostaining for avian poxvirus antigen in desquamated and superficial conjunctival epithelium. Note positive immunostaining in the cytoplasm of subconjunctival macrophages. ABC method, Mayer's hematoxylin counterstain, 200X.

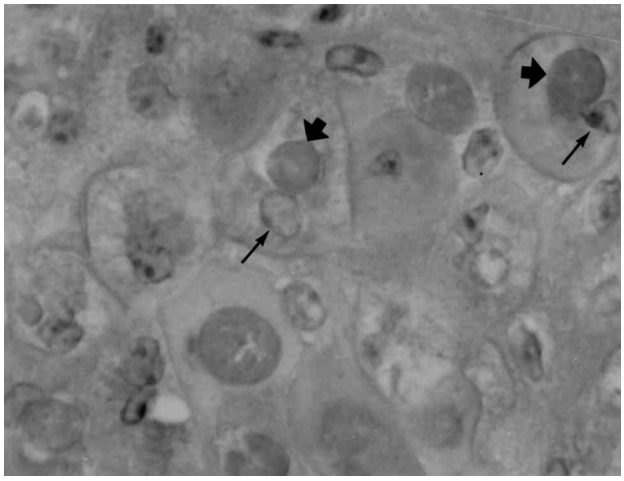

Figure 5. Positive immunostaining for avian poxvirus antigen in the cytoplasm of epidermal cells (brick-red color). Note negative immunostaining of the nuclei (thin arrows) and pale blue cytoplasmic inclusions (thick arrows). ABC method, Mayer's hematoxylin counterstain, 800X.

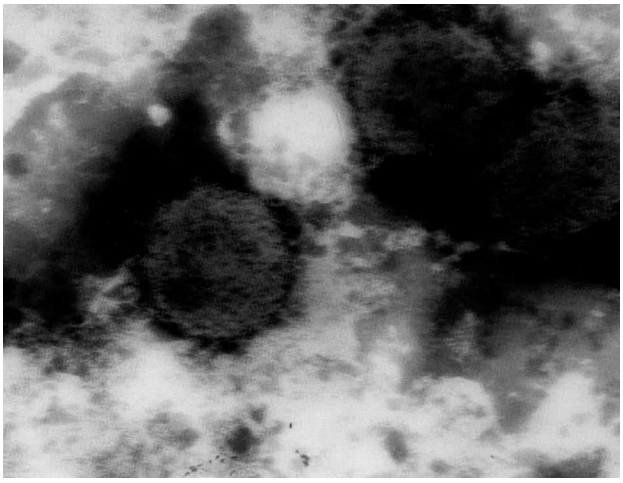

Figure 6. Viral particles consisted of a brick-shaped with a centrally placed core. Transmission electron microscope, negatively stained, 66000X.

poxvirus infection, might have resulted from emaciation due to anorexia, and difficulty in searching for food because pox lesions involving eye lesions often obstructed vision.

Poxvirus infection occurs through mechanical transmission of the virus to the injured or lacerated skin, especially by bloodsucking arthropods such as mosquitoes (3). Contact transmission via direct or contaminated objects is another route between the infected and susceptible birds (3,18). In this study, the initial source of infection and routes of infection from bird to bird was not determined, however, the presence of lesions commonly on the eye may be reflecting the conjunctival transmission from bird to bird. The virus may reach the laryngeal region via the lacrimal duct to cause infection of the upper respiratory tract (19). In the experimental

study and natural infections, the viral antigen has been detected in organs such as lungs and trachea (14). Although viral antigen was detected in the lacrimal glands of some quails, attempts to demonstrate antigen in respiratory organs were unsuccessful. For this reason, focal inflammatory cell infiltration without a detectable pox viral antigen in the lungs, spleen, liver and proventriculus might be due to systemic effects of the infection rather than direct virus-associated lesions. A secreted protein, called (vaccinia growth factor) VGF, is synthesized early in infection and binds to the epidermal growth factor receptors that induced hyperplastic responses of non-infected epithelial cells (4). Furthermore, proliferating cells without typical lesions of poxvirus had no positive immunostaining, confirming this pathogenesis.

Quailpox virus is a distinct species of the genus Avipoxviridae, and quailpox virus had no immunologic relationship to pigeon and fowlpox viruses (20,21). Poxviruses are still occasionally isolated from previously vaccinated birds and these infections are probably a reflection of the host range of antigenically distinct avipoxviruses (20). Moreover, quails vaccinated with pigeon and fowl poxviruses are not protected against challenge of their immunity with quail poxvirus (21). Immunological strain differentiation is difficult due to the presence of cross-reacting antigens, while avipoxvirus infection can be readily diagnosed by microscopic examination or virus isolation (20). In this regard, in areas where poultry is reared in close proximity to quails, cross infection is possible (20,21). According to the owner, there was no history about the presence of a pox infection in other neighboring poultry flocks and the present quails had not been vaccinated against pox infection. In this study, specific attempts were not made to characterize poxvirus propagated from the sick quail among the other avipox subgroups. However, we used antisera raised against fowlpox virus for immunohistochemistry and gel diffusion test in the detection of poxvirus antigen.

Electron microscopy is a useful technique to verify the presence of poxviruses (9). Direct electron microscopy of negatively stained preparations provides a reliable and rapid genus diagnosis but no species diagnosis of pox (1). Orf virus by direct electron microscopy was identified in scabs collected from affected lambs (22). In this research, avipoxvirus by direct electron microscopy was confirmed the presence in tissue samples of infected quails.

Tripathy et al. (23) have been detected pale to dark-brown stained intracytoplasmic inclusions in sections of formalin-fixed fowlpox-infected CAMs using peroxidase-labeled antibody. In the skin of chickens experimentally infected with fowlpox virus, the staining of the antigen was diffuse in the cytoplasm regardless of the inclusion bodies, and many cytoplasmic inclusion bodies demonstrated strong reaction on their margins by in situ hybridization and immunohistochemistry (14). In this study, no immunostaining of inclusion bodies was seen whereas the staining of cytoplasm on their margins was diffuse. It has been commonly accepted that the poxviruses replicate entirely in the cytoplasm of host cells. Early workers, however, have reported that some newly synthesized viral DNA might be associated with nucleus of poxvirus-infected cells (4). Specific and non-structural early antigens of cowpox virus reacting with monoclonal antibodies by immunocytochemistry (24), both fowl pox viral DNA and RNA by a membrane filter hybridization method (25) and sheeppox virus antigen by immunohistochemistry (9) were detected in nuclei of poxvirus-infected cells. On contrary, there was no evidence of nuclear staining in this study as well as in previous reports (14,17,26,27).

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