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Avipox Virus Infection in *Rosella parakeet* (Platycercus sp.)

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Abstract

Three parakeets were brought for postmortem examination with a history of sudden death. The gross examination of birds revealed multiple light whitish nodules around the left eye, on the skin at the level of hock joint, on the anterior part of tracheal mucosa, congested lung and pallor liver. Impression smears from nodules revealed numerous heterophils, red blood cells, necrotic epithelial cells and bacterial colonies. Histopathological examination of nodules revealed eosinophilic intracytoplasmic inclusion bodies in the spinosal cells, epidermal hyperplasia, ballooning degeneration of spinosal cells and bacterial colonies. The virus was isolated and infection was produced on both chorioallantoic membrane and chicken embryo fibroblasts. Polymerase chain reaction was carried out and primer set designed from the 4b core protein gene of fowl pox virus revealed amplification at 578 bp. Suitable remedial measures were recommended against avipox virus infection and secondary bacterial infection.

Keywords: Avipox virus infection; Rosella parakeet; Gross pathology; Histopathology; Polymerase chain reaction

Introduction

Pox is a worldwide disease caused by Avian Pox viruses (APV) belonging to the genus Avipox virus of the family poxviridae. It has been reported in numerous domestic and wild avian species. At least 232 avian species of 23 orders are sensitive to avipoxvirus infection (Bolte *et al*., 1999). The avipox virus infection was experimentally induced in eastern rosella (*Platycercus eximius*) in the florida, United States of America with the diphtheritic mucosal form (Hitchner and Clubb, 1980). There is paucity of literature on the incidence of avipox virus infection in parakeet in India. The present paper describes avipox virus infection in *Rosella parakeet* (Platycercus sp.).

Materials and methods

Three *Rosella parakeets* were brought for postmortem examination with a history of sudden death. Grossly, the carcasses revealed multiple light whitish nodules measuring about 0.5 to 2 mm in diameter around the left eye (Fig. 1), three light whitish firm nodules measuring about 0.5, 1 and 1.5 mm in diameter on the skin at the level of hock joint, light yellowish white nodules about 4 mm in diameter on the anterior part of tracheal mucosa (Fig. 2), congested lung and pallor liver. Impression smears were collected from all the nodules.

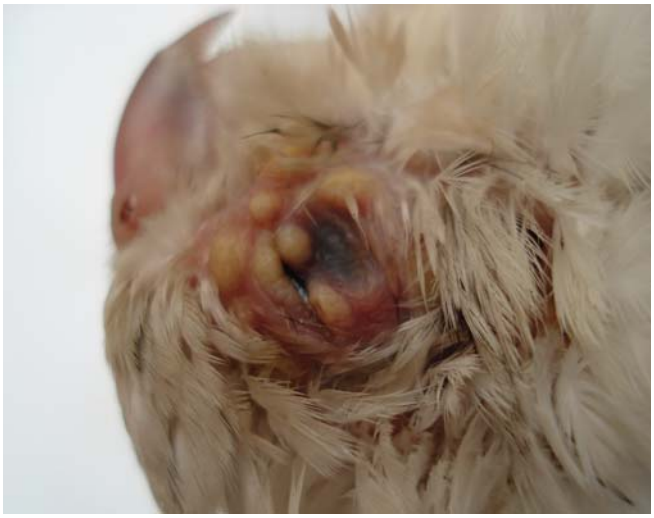

Fig.1. Avipox – Multiple light whitish nodules around the left eye.

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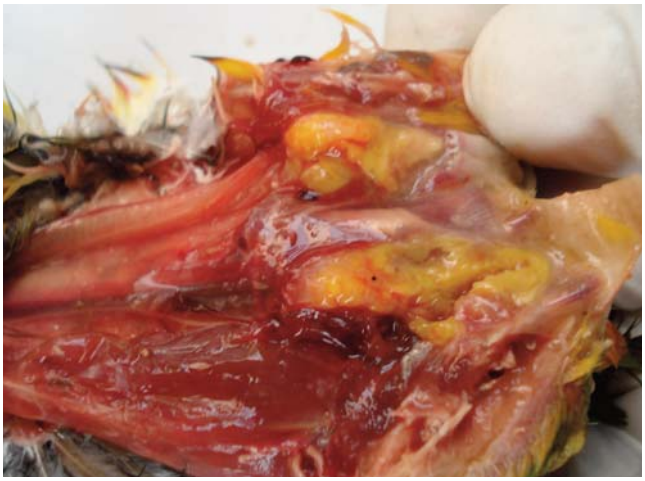


Fig.2. Avipox- Light yellowish white nodules on anterior part of tracheal mucosa.

Histopathology

The nodules were collected in 10 per cent neutral buffered formalin. The tissue samples were processed by routine paraffin embedding and 4 to 6 µm thick sections were stained with haematoxylin and eosin by employing standard procedures (Bancroft and Gamble, 2008).

Cultivation of virus

Embryonated eggs

The suspected tissue materials were processed by treating with antibiotics (Penicillin 2000U and streptomycin 200 µg) and incubated at 37°C for 30 min and inoculated in 9-11 days old embryonated eggs through chorio allantoic membrane (CAM) route. Three serial passages were carried out. The eggs were incubated at 37°C for 5 days. Eggs were chilled and CAM was harvested and pock lesions were observed only after third passage. Harvested CAM was stored at -20°C for further diagnosis (Tomar *et al*., 1988).

Chicken embryo fibroblast

The CAM material was processed and inoculated into confluent monolayer of chicken embryo fibroblasts and incubated at 37°C by using minimum essential medium (MEM-Gibco Technologies) with 10 per cent foetal calf serum.

Viral DNA isolation

The CAM obtained from third passage with sufficient number of membrane thickening and pock lesions were used for viral DNA isolation using the Qiagen DNeasy tissue kit. Viral DNA was isolated from infected CAM as described by Eposito et al. (1981). In brief, infected cells were clarified by centrifugation at 551 xg for 10 min. The supernatants were resuspended and centrifuged at 21,525 x g for 60 min, and the pellets were resuspended in TE (10 mM Tris - 1 mM EDTA). The pellets were further suspended in 0.5% 2- mercaptoethanol (2-ME) and 10% Triton X-100. The suspension was agitated on ice for 10 min and centrifuged at 1,240 x g for 5 min; the resulting supernatant was centrifuged at 31,000 x g for 60 min at 4°C. The pellet was reconstituted in TE buffer containing; 0.25% 2-ME, Proteinase K (10 mg/ml) and 20% N-lauryl sarcosinate. After 30 min agitation on ice, 54% of sucrose in water and 20% sodium dodecyl sulfate (SDS) were added and incubated at 55°C for 2 h. The resulting mixtures were extracted twice with 25:24:1(vol/vol) phenol:chloroform:isoamyl alcohol and once with 24:1 (vol/vol) chloroform: isoamyl alcohol and concentrated by ethanol precipitation.

PCR amplification

The primers were procured from Sigma Aldrich, USA, on the basis of published DNA sequence of the 4b gene sequence of FPV (HP 444) (Binns *et al*., 1989; Tomley *et al*., 1988). The primer sequence for the upper and lower ologonucleotides from 5' to 3' (Tadese and Reed , 2003) is as follows.

Core protein gene (sense) 5'-CAGCAGGTGC-TAAACAACAA-3'

Core protein gene (antisense) 5'-CGGTAGCT-TAACGCCGAATA-3'

Polymerase chain reaction was performed in a Thermocycler (Applied Biosystems). The reaction was carried out in a final volume of 30 µl containing 10X PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each deoxynucleotide, I µM of each primer, and 2.5 units of Taq DNA polymerase (Finnzyme). The reaction mixture was subjected to 35 amplification cycles. Each cycle consisted of a hot start at 94°C for 7 min, denaturation at 94°C for 30 sec, annealing at 50°C for 60 sec and extension at 72°C for 7 min. PCR products were separated on 2 per cent agarose gel in 1X 89mM Tris-borate-89 mM boric acid-2mM EDTA (TBE).

Results

Impression smears collected from nodules revealed numerous heterophils, red blood cells, necrotic epithelial cells and microorganisms indicating secondary bacterial infection.

Histopathological examination of the nodules revealed characteristic eosinophilic intracytoplasmic inclusion bodies in the spinosal cells called Bollinger bodies (Fig. 3). Besides, the epidermis showed hyperplastic changes, ballooning degeneration was noticed in the spinosal cells. Basophilic staining colonies of bacterial rods were observed at the surface of the epidermis.

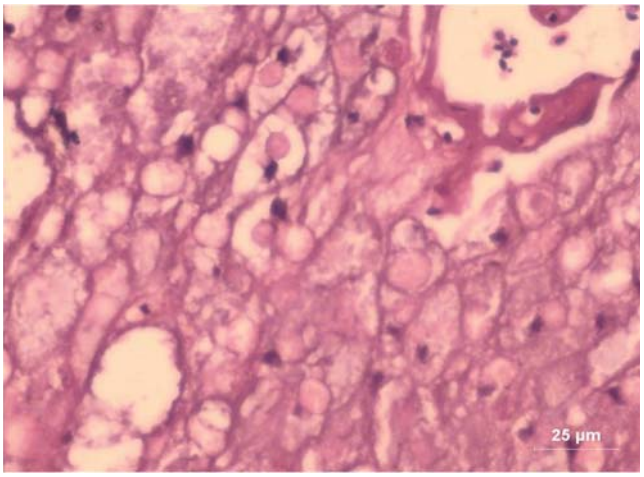


Fig..3. Avipox- Eosinophilic intracytoplasmic inclusion bodies. Scale bar H&E 25 µm.

Cultivation of virus

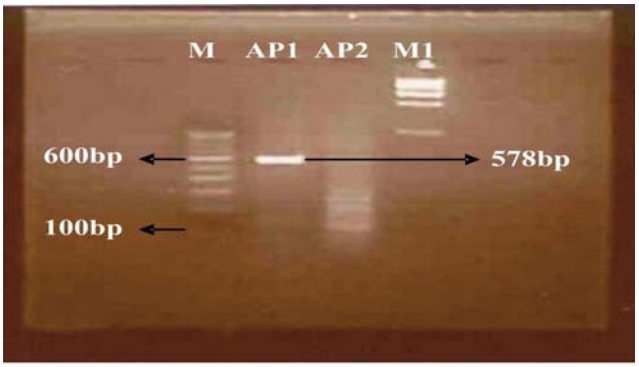
Initial two passages in the embryonated eggs produced only a few thickenings in the CAM. The third passage revealed multple thickened areas and pock lesions.

Chicken embryo fibroblast

In chicken embryo fibroblasts, rounding and detachment of cells were observed 48 h post infection. Spiderweb pattern of cytopathic effect characteristic for fowlpox was observed 96 h post infection.

Polymerase chain reaction

A primer set designed from the 4b core protein gene of FPV amplified a 578 bp product in the samples (Fig. 4) which confirmed fowl pox virus.



M – 100bp Molecular weight marker
AP1 – Avipox sample 1
AP2 – Avipox sample 2
M1 – 1kb Molecular weight marker

Fig..4. Amplification of DNA at 578 bp.

Discussion

Avipox virus infection had two forms viz., cutaneous and diphtheritic forms. Tripathy and Reed (2003) reported that gross lesions in both the cutaneous and diphtheritic/pharyngeal forms were usually sufficient to suspect a pox infection.

Histopathological examination of nodules in this case revealed eosinophilic intracytoplasmic inclusion bodies which was suggestive of avipox virus infection. The histological features are regarded as definitely confirmatory for the diagnosis in any affected species (Pass, 1996).

Avian pox virus infection has also been confirmed by serologic tests, such as the agar gel precipitation test and passive haemagglutination test. These detection methods have considerable limitations because of cross reactions and cannot differentiate serologic responses of different species or strains of pox virus (Tadese and Reed, 2003). Polymerase chain reaction has been one of the most extensively applied techniques in the differentiation of various species and strains of viruses (Herman *et al*., 1994, 1997; Long and Kuo, 1997).

In the present case, 4b core protein gene of FPV amplified a 578 bp product which is in agreement with earlier report of Tadese and Reed (2003).

Mortality percentage was 7.5 as three out of 40 birds died. Suitable remedial measures were recommended to control avipox virus infection in other birds and secondary bacterial infections by using ciprofloxacin at the dose rate of 1 g /1 liter of water for 5 days. No further mortality was recorded.

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