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Incidence, pathology and diagnosis of fowlpox, pigeonpox and duckpox in Asom*

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ABSTRACT

A study was conducted to see the incidence of avian pox in different districts of Asom and one district of Nagaland, where outbreaks, 29 of fowlpox, 13 of pigeonpox and 3 of duckpox were recorded. The age wise morbidity and cause specific mortality in case of fowlpox was recorded at 0–8 weeks (19.63 and 23.60%), at 9–20 weeks (5.57 and 14.43%) and above 20 weeks (1.39 and 11.11%), respectively. Likewise, in pigeonpox, morbidity and cause specific mortality were recorded at 0–8 weeks (11.11, 0%), 9–20 weeks (30.68, 37.03%) and above 20 weeks (35.08, 30%), respectively. Again in case of duckpox, morbidity was recorded at 0–8 weeks (10%), 9–20 weeks (0%) and above 20 weeks (6.66%), while no mortality was recorded among the ducks. External examination revealed erosions, crusts and several small, multifocal to coalescing wart-like nodules on various parts of the affected birds. During post-mortem examination, few birds showed fibronecrotic lesions on mucous membrane of the oral cavity and upper respiratory tract. Histopathological examination of the scab samples revealed intracytoplasmic eosinophilic inclusion bodies. During ultrastructural study, inclusion bodies were seen in the cytoplasm of the skin epithelium, which consist of numerous, dumbbell-shaped bodies typical of pox virions. During molecular diagnosis, out of 29 fowlpox, 9 pigeonpox and 3 duckpox suspected samples 86.20, 77.77 and 100% samples, respectively, were found positive by polymerase chain reaction.

Key words: Duckpox, Fowlpox, Inclusion body, Pigeonpox, Polymerase chain reaction

Avian pox is an infectious and contagious viral disease where poultry, pet and different wild birds are commonly affected. Among different avipoxviruses of domestic birds, fowlpox virus (FPV) and pigeonpox virus (PPV) infection is found to be very common; whereas, records related to the incidence of duckpox (DP) are scanty. However, efforts have been made to update the available literature. Zheng *et al.* (2015) isolated a novel duck-pathogenic avipoxvirus from domestic mallard ducks of China. From the available literature it was observed that work done on fowlpox (FP), pigeonpox (PP) and DP in Asom related to its molecular and ultra structural study is meagre and requires further study. Therefore, an attempt was made to work on incidence of FP, PP and DP in different districts of Asom and only one district of Nagaland during 2014 to 2016.

MATERIALS AND METHODS

Samples from pox infected fowl, pigeon and ducks were collected from various organized farms, local markets as well as from household birds. When a suspected bird was found, the detailed clinical history, gross alterations were recorded. Clinical samples like scab materials were collected from suspected cases from dead and sacrificed birds at the time of post-mortem examination. All the visceral organs were thoroughly examined and samples like pieces of heart, lung, liver, kidney, oesophagus and scab were collected in 10% formaline solution. Scab materials and affected parts suspecting diphtheritic form of pox were also collected aseptically in sterile vials for isolation and PCR and stored at -20°C until further use. Paraffin embedded tissue sections were stained with Haematoxylin and Eosin (H and E) method (Luna 1968).

Ultrastructural study: For ultrastructural study, the affected scab materials were cut into small pieces and fixed in 2.5% gluteraldehyde. The fixed tissue samples were placed in 0.1 M Karnovsky's buffer for 15 min at 4°C and this step was repeated 3 times. After that, the tissue samples were submitted to the Sophisticated Analytical Instrumentation Facility (SAIF), NEHU, Shillong, Meghalaya for further processing (Wischnitzer 1971).

Isolation of virus: The various field samples of FP and PP were adapted on chorioallantoic membrane (CAM) of embryonated chicken eggs free from avipox specific maternal antibody up to 5 serial passages. Duckpox samples were adapted on CAM of embryonated duck egg up to 5

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serial passages. Passaged materials were stored at –20°C for future use.

Confirmation of avipoxvirus by PCR: PCR was performed to detect the pox infection either from the tissue samples (wart) or from the CAM of embryonated chicken/ duck eggs obtained during isolation. The DNA was extracted by DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's guidelines.

The PCR was performed with slight modification using a primer pair described by Lee and Lee (1997) based on 4b sequence of fowlpox virus strain HP444 as:

Forward primer: 5'-CAGCAGGTGCTAAACAACAA-3'
Reverse primer: 5'-CGGTAGCTTAACGCCGAATA-3'

For conducting PCR, 25 µl reaction mixture was prepared by 3 µl DNA template with 12.5 µl master mix (2×), 1 µl species specific primer of both forward and reverse and made the volume up to 25 µl with nuclease free water. The PCR tube containing the mixture were tapped gently and quickly spun at 8,000 rpm for 3–5 sec. The reaction mixture was subjected to amplification in a thermocycler (Applied Biosystem, USA) with following reaction condition (Table 1).

Table 1. Thermal cycling condition for APV-specific PCR

Initial denaturation	At 94°C for 5 min	
Denaturation	At 94°C for 30 sec	× 35 cycles
Primer annealing	At 60°C for 1 min	
Elongation	At 72°C for 1 min	
Final elongation	At 72°C for 5 min	

The amplified PCR product of 4b core gene was confirmed by agarose gel electrophoresis in 1× Tris Acetate EDTA (TAE) buffer with 1.2% agarose (Amresco) containing ethidium bromide (10 mg/ml). Electrophoresis was carried out at 80V for 1 h and amplified product was visualized at 312 nm wavelength as a single compact band of expected size under UV light using a UV transilluminator.

RESULTS AND DISCUSSION

In the present investigation, 29 FP outbreaks were recorded. The district wise morbidity and cause specific mortality percentage were recorded as Kamrup (Metro) (8.06, 29.78), Lakhimpur (24.24, 25.00), Kamrup (Rural) (15.65, 20.37), Baksa (BTAD) (7.72, 18.60), Nalbari (8.09, 18.42), Dibrugarh (3.28, 8.00) and Phek (Nagaland) (12.29, 28.84). Among different age groups, highest morbidity and cause specific mortality were recorded at 0–8 weeks (19.63% and 23.60%) followed by 9–20 weeks (5.57% and 14.43%) and above 20 weeks (1.39% and 11.11%). The total cause specific mortality was 20.74% during the study. In two farms of Kamrup (Metro) district, heavy infection was recorded in 20–25 days old chicks. The highest incidence of FP at 0–8 weeks was probably because of absence of maternal antibody in the chicks, as vaccination against FP was not done in poultry regularly. Our findings concorded with the observation made by Sawale *et al.* (2012) who investigated eight farms and diagnosed FP as cause of death at the age group ranged from 25 days to 29 weeks.

Similarly, during the study, 13 PP outbreaks were recorded where district wise morbidity and cause specific mortality percentage were Kamrup (Rural) (34.37, 36.36), Kamrup (Metro) (32.05, 36.00), Nalbari (28.57, 25.00), Baksa (BTAD) (21.05, 25.00) and Barpeta (16.66, 0). The age wise morbidity and cause specific mortality were recorded at 0–8 weeks (11.11%, 0%), 9–20 weeks (30.68%, 37.03%) and above 20 weeks (35.08%, 30.00%). The total cause specific mortality was recorded as 32.65%. In comparison to FP, the incidence of PP was highest in grower and adult groups. As the disease is prevalent among the grower and adult birds so there may be presence of antibody among these birds which may be carried to their squabs through eggs and this may be the reason of getting very low incidence of PP in 0–8 weeks of pigeons.

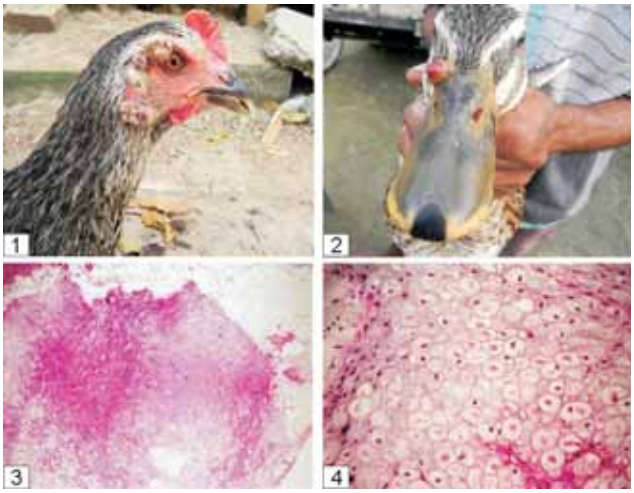
In the present investigation, only four clinical cases of DP were recorded from three outbreaks where two ducks were of 43 days old and the rest two were adult ducks. The district wise morbidity percentage were Kamrup (Metro) (10), Baksa (BTAD) (6.25) and Nalbari (3.12). Among the age groups, highest morbidity was recorded at 0–8 weeks (10.00%) followed by above 20 weeks (6.66%) and at 9–20 weeks (0%), while no mortality was recorded among the affected ducks. Regarding DP occurrence, Zheng *et al.* (2015) expressed the possibility that avipoxvirus has been recently introduced by wild waterfowl into domestic mallard ducks and further study is needed to determine the pathogenecity of the virus on other poultry species.

In the present investigation, the mortality in all the species recorded were very low which was in accordance to the findings of Tripathi and Reed (2008) who stated that low mortality occurred in uncomplicated cases of FP.

Clincal signs and gross pathology: Clinical examination of some of the affected fowls, pigeons and ducks showed anorexia, weight loss, stunted growth, decrease of egg production, alopecia of feathered skins with yellow scabs, respiratory distress and depression. Due to involvement of eyes, some of the affected birds showed unilateral or bilateral blindness owing to blepharitis and conjunctivitis.

External examination of the affected fowls, pigeons and ducks revealed erosions, crusts and several small, multifocal to coalescing wart-like nodules on the comb, wattle, eyelids, head, legs, thighs, eyes, wings, vent, beak, bill and neck region (Figs 1, 2) which were similar as reported by various workers (Sawale *et al.* 2012, Pandey *et al*. 2014, Okwor *et al.* 2014). Similarly, diphtheritic form of pox was also observed in some birds where internally fibronecrotic lesions on mucous membrane of the oral cavity and upper respiratory tract were noticed.

During post-mortem examination of dead birds, no remarkable gross lesions were seen in various visceral organs which was in agreement with Gulbahar *et al*. (2005) and Sawale *et al*. (2012). Relating to these findings, Vegad (2004) stated that some pox viruses enter into the blood of



Figs 1–4. **1.** Lesions in fowlpox. **2.** Lesions in duckpox. **3.** Hyperplasia of the epithelial cells of the epidermis with eosinophilic intracytoplasmic inclusion bodies and infiltration of polymorpho-nuclear and mononuclear cells. (H and E ×100). **4.** Vacuolation of hyperplastic epidermal cells with ballooning degeneration and eosinophilic intracytoplasmic inclusion bodies.

affected birds and cause viraemia without showing any change in visceral organs.

Histopathology: The skin lesions collected from the scabs revealed varying degrees of hyperplasia of the epithelial cells of the epidermis and feather follicles. The hyperplastic cells were vacuolated with ballooning degeneration. Most of the cells contained large eosinophilic intracytoplasmic inclusion bodies (Bollinger body). In some birds, epidermis showed edema, congestion and necrosis along with infiltration of moderate to severe polymorphonuclear and mononuclear cells (Figs 3, 4).

In the lungs, peribronchial lymphoid nodule formation, capillary congestion along with fibrinous exudates and polymorphonuclear and mononuclear cells in the bronchial lumen were observed in few birds. Epicardial congestion in the heart, sinusoidal congestion, fatty changes and hydropic degeneration in the liver, sub-capsular aggregation of mononuclear cells and focal inter-tubular congestion in the cortical areas of the kidneys were the histopathological changes observed in some of the affected birds. Although APV 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 was reported by Beytut and Haligor (2007).

Ultrastructural study: During the study, inclusion bodies were seen in the cytoplasm of the skin epithelium which consists of numerous dumbbell-shaped bodies typical of pox virions. Each virion consisted of a brick-shaped structure with an envelope, an electron dense centrally located core and two lateral bodies (Fig. 5) which was in agreement with the findings of Nakamura *et al.* (2006) and Beytut and Haligor (2007).

Molecular detection of APV: All the positive samples showed specific bands of 578 bp in 1.2% agarose gel

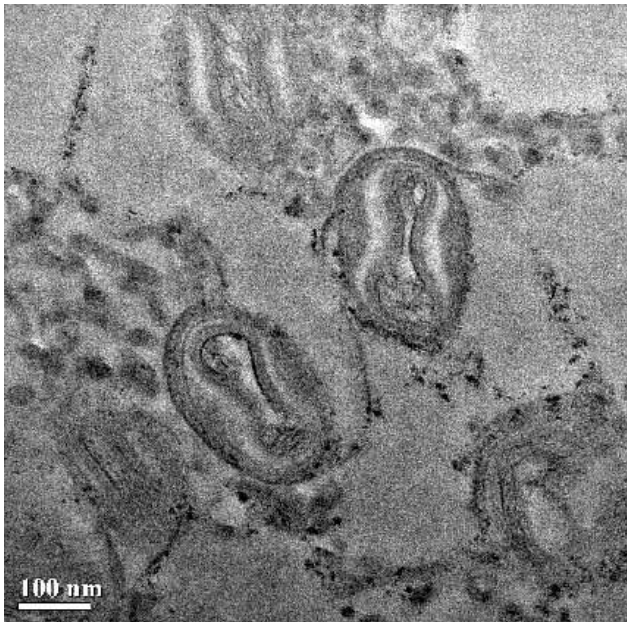


Fig. 5. Typical dumb-bell shaped poxviral particles showing envelope, centrally located core and two lateral bodies (skin) (TEM).

electrophoresis against no band in negative control (Fig. 6).

Out of 29 FP, 9 PP and 3 DP suspected samples; 25 (86.20%) FP, 7 (77.77%) PP and 3 (100%) DP samples were found positive by PCR. The reason for the negative results of the remaining samples might be due to selection of inappropriate tissue for DNA extraction. Similarly by using PCR, Fahmy *et al.* (2009), Roy *et al*. (2013) and Zheng *et al*. (2015) also diagnosed pox in pigeon, fowl and duck. In this study, it was confirmed that primer sets used for FP recommended by Lee and Lee (1997) also amplified the PP and DP.

In conclusion, the present study indicated that among avian pox, FP, PP and DP outbreaks are occurring very frequently in North-Eastern part of India and incidence of FP is seen very high in young birds. Moreover, occurrence

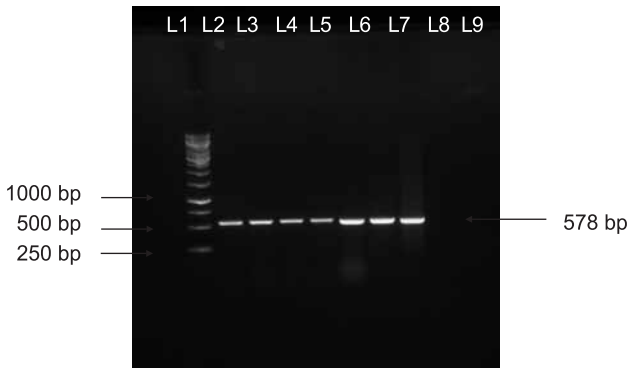


Fig. 6. Agarose gel electrophoresis showing amplicons. Lane 1, Ladder (1 kb); lane 2, positive control (fowlpox); lanes 3–4, fowlpox field virus isolates; lanes 5–6, pigeonpox field virus isolates; lanes 7–8, duckpox field virus isolates; lane 9, negative control (no sample loaded).

of DP is an alarming situation in this region. So rapid and specific diagnosis and development of vaccine is the need of the hour.

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