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RESEARCH ARTICLE

PATHOLOGY AND MOLECULAR DIAGNOSIS OF FOWL POX IN SHILLONG, MEGHALAYA

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ABSTRACT

A study was conducted during the period from August, 2015 to April, 2016 to survey the occurrence of viral diseases in chicken in and around Shillong, Meghalaya, to study the pathology and finally to diagnose them by using common molecular techniques. A total of 370 dead and sick birds were collected from different organized and unorganized poultry farms in and around Shillong, Meghalaya. Of these, 109 cases (i.e. 29.46%) were diagnosed as viral diseases. Only 5 cases out of 21 clinically suspected cases could be diagnosed as Fowl pox (1.81%) basing on the clinical history, gross and histopathology and confirmed by PCR. The maximum cases were mostly in birds of 9-12 weeks (40%), followed by 3-9, 9-12 and above12 weeks of age (20%) with very low morbidity (5-7%). The general symptoms recorded were depression, dehydration, emaciation, and reluctance to move due to wart-like growth on the eyelid impeding their vision. In some chickens, egg production was affected. There were no significant gross lesions except for the wart-like nodules, crust and erosions on featherless parts. Most characteristic microscopic changes were hydropic degeneration and hyperplastic epithelium of stratum spinosum with presence of pathognomic eosinphillic intracytoplamic inclusion bodies called Bollinger bodies. There were areas of congestion and necrosis under the suferficial layer of skin. PCR was also used for confirming the disease by detecting the viral genome (i.e. 4b gene). The present study suggests that fowl pox is occurring in chicken population in and around Shillong, Meghalaya.

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INTRODUCTION

Fowl pox or avian pox is an infectious, contagious viral disease that may infect virtually any order of birds, either wild or domesticated (Tripathy, 2008; Silva *et al*., 2009). The disease has a world-wide distribution and is caused by a double stranded DNA virus of the genus Avipoxvirus, subfamily Chordopoxvirinae of the family Poxviridae (Fauquet *et al*., 2005; Chambers, 2009). The disease can occur as a mild cutaneous form (dry pox) characterized by the development of proliferative lesions, ranging from small nodules to spherical wart-like masses on the skin of the comb, wattle and other unfeathered areas with low mortality or as a diphtheritic form (wet pox) which can be more severe or both (Ariyoshi *et al*., 2003; Adebajo *et al*., 2012). The mortality rate is higher in the diphtheritic form than in the cutaneous form, sometimes nearing 50% particularly in young birds (Singh *et al*., 2008; Singh *et al*., 2003).

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Clinical signs observed with avian pox are weakness, emaciation, difficulty in swallowing and breathing, vision problems, a reduction in egg production soiled facial feathers, conjunctivitis and edema of the eyelids and the presence of the characteristic wart-like growths on the unfeathered portions of the skin (Jordan *et al*., 1996). In diphtheritic form wart-like lesions can be seen in the diphtheritic membrane on the upper portion of the digestive tract. Proliferating lesion involving the nasal passages, larynx or trachea can result to respiratory distress and death from suffocation (Afonso *et al*., 2000; Tripathy *et al*., 2000). Histologically, the cytoplasm of the hyperplastic epithelial cells in the infected skin and respiratory tract mucosa contain characteristic large eosinophilic inclusions (identified as Bollinger bodies) and vacuoles (Beytut, 2007). Lesions are characterized by the presence of epidermal hypertrophy and hyperplasia in cutaneous lesions, with ballooning degeneration of stratified squamous epithelium. Epithelial cells are commonly swollen, rounded, and separated from each other in the stratum spinosum layer. The superficial epidermis of the lesions is ulcerated with eosinophilic, amorphous keratinaceous crusts, necrosis, and

numerous degenerated granulocytes. In the diphtheritic form of the disease, nodular hyperplasia (increase in the number of cells) of the mucosa is observed (Smits *et al*., 2003). A presumptive diagnosis of avian pox can be made due to the gross lesions on the body. Confirmation of avian pox is accomplished by microscopic examination for the characteristic Bollinger bodies. Virus isolation by transmission of the organism via egg inoculation, serological results and polymerase chain reaction can also be a means of confirming the disease (Oros *et al*., 1997; Rocke *et al*., 2005; Hess 2011).
Both organized and unorganized poultry farms in and around Shillong, Meghalaya were visited regularly during the study period from August, 2015 to April, 2016 and the morbidity, mortality, age of affection of various diseases were recorded. To assess the age-wise variations in the incidence of the diseases, the birds were grouped as 1-3, 3-6, 6-9, 9-12 and above 12 weeks old. In case of mortality/outbreak of diseases in the poultry population, the clinical signs exhibited by the individual bird during illness were recorded in details according to the description of the respective poultry farm's owner or attendant. In addition, sometimes some sick/moribund birds were kept under careful observation with feed and water *ad libitum* till death to record the detailed clinical signs along with other abnormalities.

Detailed post-mortem examination of all the dead birds was performed and gross tissue changes were recorded carefully. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, brain, feather follicles, etc.) showing lesions were carefully collected in 10% formaldehyde solution for histopathological examination. These were processed and stained with Mayer's hematoxylin and eosin (Bancroft, 1980). The diagnosis of the disease was made mainly basing on the clinical signs, characteristic gross and microscopic changes. Confirmation of the disease was accomplished by microscopic examination for characteristic Bollinger bodies. In addition, polymerase chain reaction (PCR) was also used for confirming the disease. PCR amplification of 4b protein encoding gene was performed using specific primers (Binns *et al*., 1989). Tissue samples from affected skin (wart-like nodules, crust and erosions on featherless parts of body) of a total of 21 clinically fowl pox suspected cases were tested for detection of the 4b gene. In the present investigation, the disease was observed to affect almost all age groups except the young chickens of 1-3 weeks old, as similarly observed by earlier researcher (Jarmin *et al*., 2006).

Fig.1. FP affected birds showing reluctance to move, anorexia & depression

It was found to occur all around the year as described by previous workers (Pattison *et al*., 2008). Mortality percentage recorded was very low as the cases were of all cutaneous forms of the disease which caused less severity. Not a single case of diphtheritic form was found during the period of study. The affected chickens were anorexic, depressed and reluctant to move due to wart-like nodular growths on the eyelid impeding their vision (Fig. 1). In some birds, egg production was affected. Most of the birds showed weakness, emaciation, difficulty in swallowing and breathing, vision problems, a reduction in egg production, soiled facial feathers, conjunctivitis and edema of the eyelids and the presence of the characteristic wart-like growths on the unfeathered portions of the skin. Similar clinical signs have been described by earlier workers (Jordan, 19996).

Fig. 2.Wart-like nodular growths on the face, beak and eyelids

Fig.3. Wart-like nodular growths on the face, beak and eyelids

The main gross lesions recorded during the present study period were the wart-like projections (nodular growths) which were rough, large after coalescing, brown to grey colour on the face, eyelids and beaks (Figs. 2 and 3), which were characteristic for cutaneous form of the disease. Similar lesions have also been described by some researchers (Yoshikkawa, 2002; Riper, 2006). However, characteristic lesions for diphtheritic form of the disease were not observed in any of the affected birds. Similarly, some workers (Khan *et al*., 2009) also showed dry pox lesions on the external body surfaces of peafowl with no internal lesions in necropsied birds. Some birds had prominent keel bones during post-mortem examination which might be due to starvation. Microscopic examination of the skin sections revealed hydropic degeneration and hyperplastic epithelium of stratum spinosum (Fig. 4).

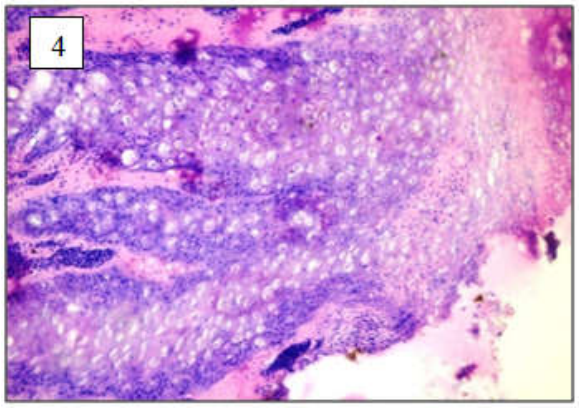

Fig.4: Hydropic degeneration and hyperplastic epithelium of stratum spinosum (H&E, 20X)

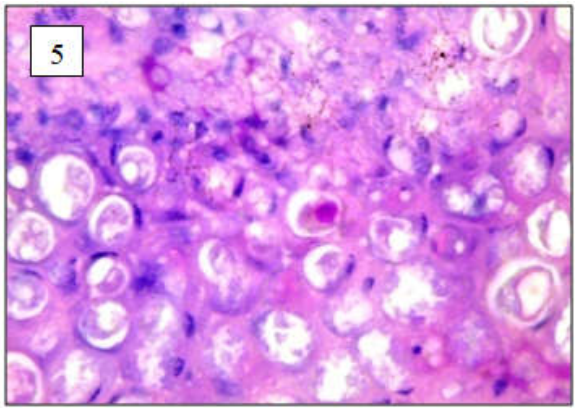

Fig.5. Hyperplastic epithelial cells showing hydropic degeneration and eosinophillic inclusion bodies (H&E, 100X)

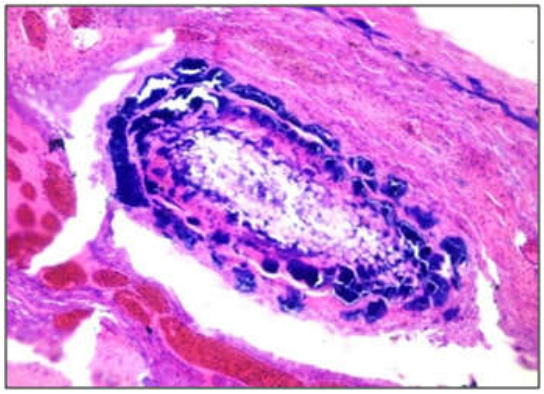

Fig.6. Congestion & necrosis in superficial layer of skin (H&E, 40X)

The hyperplastic epithelial cells showed hydropic degeneration (ballooning degeneration) and presence of large eosinophillic inclusion bodies - Bollinger bodies (Fig. 5). In most of the cases, there was congestion and areas of necrosis in superficial layer of skin (Fig. 6). These microscopic changes are in agreement with the findings of many workers (Beytut, 2007; Smits *et al*., 2003; Gortazar *et al*., 2002; Gulbahar *et al*., 2005). Presumptive diagnosis was made by the presence of gross lesions (wart-like nodules) on the unfeathered parts of the body. Confirmation of the disease was accomplished by microscopic examination for characteristic Bollinger bodies. In addition, polymerase chain reaction (PCR) was also used for confirming the disease. Several researchers also used similar techniques (Rocke *et al*., 2005; Ganesh *et al*., 2002; Kumar *et al*., 2010; Susan *et al*., 2014; Zheng *et al*., 2015).

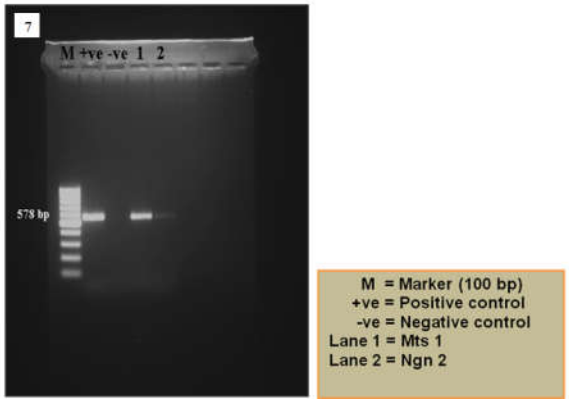

Fig.7. 1.5% Agarose gel electrophoresis stained with Ethidium bromide showing the PCR products (578 bp) of Fowl pox virus in tissue samples

Out of 21 fowl pox suspected cases, 5 cases were found positive for the viral genome. A clear and distinct band of PCR product was appeared at the position of 578 bp with the standard 100 bp DNA ladder passed through 1.5% Agarose gel electrophoresis (Fig. 7).

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