



## RESEARCH ARTICLE

### STUDIES ON OCCURRENCE AND PATHOLOGY OF VIRAL DISEASES IN CHICKEN IN AND AROUND SHILLONG, MEGHALAYA

Amlyne G. Momin and \*Damodar Singh, Y.

Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram-796014 (India)

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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. The diseases were diagnosed based on clinical history, clinical signs/symptoms prior to death, gross lesions observed on post-mortem examination, histopathology and laboratory detection of viral genomes. Among the diseases, Newcastle disease (ND) was found in 15.14%, followed by infectious bursal disease (IBD) in 12.97% and fowl pox (FP) in 1.81%, respectively. Age-wise analysis showed that the maximum number of cases were recorded in the age group of 3-6 weeks (33.03%), followed by 6-9 weeks (28.44%), 9-12 weeks (17.43%), 1-3 weeks (14.68%) and above 12 weeks (6.42%) age groups of chicken. The present study suggests that some important viral diseases are occurring in chicken population in and around Shillong, Meghalaya with higher incidence in 3-6 weeks age groups.

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## INTRODUCTION

Poultry industry has made remarkable progress in Meghalaya during the last few decades, from a backyard venture to a considerable sophisticated commercial industry. Total poultry population of Meghalaya was estimated to be 35,65,059 as per 19<sup>th</sup> Livestock Census, 2012 (Anonymous, 2012). However, a negative trend in the growth was observed in last few years. Egg consumption in Meghalaya is about 38 numbers per head per year which is more than all India level of 30 eggs, but far below the minimum recommended levels. One of the most important reasons behind this is frequent incidence of diseases in poultry farm leading to huge economic losses to the poultry farmers. The poultry health care system in Meghalaya suffers due to difficult geographical terrains which lead to reduced accessibility, weaker linkages and a poor health delivery system. In rural areas, diagnosis of poultry diseases is mostly based on clinical and post-mortem examination. Often the etiology of outbreak of diseases, which cause huge loss to the poultry farmers, remains undiagnosed. The inadequate measures adopted by the stakeholders for the control of

diseases in addition to inefficient management have rendered the poultry farming a risky business. Poor reporting system and lack of mass surveillance facilities are also among the major constraints for generating the data regarding exact disease status and the economic losses. However, no detailed studies have been done on poultry diseases in this area so far. So, the epidemiology, pathogenesis and pathology of the poultry diseases in this area are not fully known.

## MATERIALS AND METHODS

The epidemiological data pertaining to viral diseases in poultry from August, 2015 to April, 2016 were collected from both organized and unorganized poultry farms in and around Shillong, Mizoram. Detailed information such as total birds in a flock, number of birds affected, number of birds died, age of the affected birds, month of occurrence of the disease, history of previous outbreaks of viral diseases and vaccination status were obtained from the affected flocks. Dead/moribund birds were collected for proper necropsy. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, etc.) showing typical lesions were collected for histopathological examination and laboratory analysis. Both organized and unorganized poultry farms in and around Shillong, Meghalaya were visited

\*Corresponding author: Damodar Singh, Y.

Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram-796014 (India).

regularly 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 detail in a prescribed 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. At necropsy, gross tissue changes were observed and 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 ice and in 10% formaldehyde solution. Viable tissue samples were collected aseptically in sterile polypropylene zipper bags and stored in -80°C for further analysis.

Formalin fixed tissues (2-3 mm thick) were taken, washed overnight in running tap water and then dehydrated in ascending grades of alcohol starting from 50%, 70%, 90% and absolute alcohol I, alcohol II, alcohol III and finally cleared in xylene. These dehydrated tissue pieces were then embedded in molten paraffin. Sections were cut at 4-5 µm thick with semi-automatic rotary microtome (MRS 3500, Histoline Laboratories) and stained with Mayer's hematoxylin and eosin (Bancroft and Stevens, 1980). The stained slides were examined under a trinocular research microscope (Olympus) and the magnified images of the tissue structures were captured for further study. The diagnosis of the diseases was made mainly basing on the clinical history, signs, characteristic gross lesions and microscopic tissue alterations. However, PCR or RT-PCR assays were employed to confirm the diagnosis by detecting the viral antigens of diseases like infectious bursal disease (IBD), Newcastle disease (ND) and fowl pox.

*et al.*, (2009). Fowl pox with a proportionate incidence of 1.81% was low in comparison with ND and IBD, which was similarly reported by Dolka *et al.*, (2012), but lower than that of Ukashatu *et al.*, (2012). Age-wise analysis showed that the highest occurrence of viral diseases in the age group of 3-6 weeks (40.38%), which supports the earlier reports of Rahman and Samad (2005). Name of the viral disease, total number of cases examined, age of birds, number of confirmed cases and proportionate incidence% recorded during the present study period are shown in (Table 1).

### Newcastle disease (ND)

During the present study period, the disease was found to affect all age groups of birds as similarly described by (Shankar, 2008). Maximum cases of the disease were recorded in 6-9 weeks old chickens (30.71%), which support the findings of Olabode *et al.*, (2012). The percent morbidity (35-50%) and percent mortality (25-35%) recorded during the present study indicates that there might be involvement of virulent NDV in these outbreaks. Common clinical signs recorded during the present study included emaciation, depression, laboured breathing with other respiratory signs such as coughing, sneezing, and nasal discharge. In most of the birds, the lower eyelids were congested and swollen leading to conjunctivitis. Greenish or whitish diarrhea was a frequent finding. Face, head including the wattles showed oedema in some chicken. Nervous signs like torticollis and paralysis were also observed. These findings are almost similar to those of many researchers (Beard and Hanson, 1992; Gowda and Eswaran, 1992; Bhaiyat *et al.*, 1994; Capua *et al.*, 2002; Shankar, 2008; Khan *et al.*, 2011; Nidzworski *et al.*, 2013). Some layer birds were seen to lay soft shelled eggs with severe drop in egg production, which had been similarly described by Shankar (2008), Hadipour *et al.*, (2011) and Nidzworski *et al.*, (2013). Many birds were found dead suddenly with few or no symptoms, which supports the description of Calnek (1991).

**Table 1. Overall occurrence of viral diseases of chicken in and around Shillong, Meghalaya: age-wise distribution and proportionate incidence**

Name of disease	Number of Carcasses Examined	Number of suspected cases	Age of birds (weeks)					Number of confirmed cases	Proportionate incidence%
			1-3	3-6	6-9	9-12	>12		
ND	370	87	7 (12.50%)	12 (21.43%)	20 (35.71%)	11 (19.64%)	6 (10.71%)	56	15.14
IBD		75	9 (18.75%)	23 (47.92%)	10 (20.83%)	6 (12.50%)	-	48	12.97
FOWL POX		21	-	1 (20.00%)	1 (20.00%)	2 (40.00%)	1 (20.00%)	5	1.81
TOTAL	370	183	16 (14.68%)	36 (33.03%)	31 (28.44%)	19 (17.43%)	7 (6.42%)	109	29.46

## RESULTS AND DISCUSSION

A total of 370 chicken carcasses were examined, of which 109 (29.46%) cases were diagnosed as viral diseases. This present percent proportionate incidence is found little higher than that of Rahman and Samad (2005) who recorded 22.73% as viral diseases. The incidence of Newcastle disease (15.14%) was found to be the highest followed by infectious bursal disease (12.97%), which is in accordance with the findings of Dey

The most common gross changes recorded during the investigation were pin point hemorrhages at the tips of the proventricular glands, while in few cases there were congestion, petechiae and ecchymoses in the mucosa of the proventriculus. Haemorrhagic necrotic ulcers and enteritis with exudates were also observed in the intestines, while haemorrhages and necrotic lesions in caecal tonsils were also recorded in many cases. Similar lesions have been reported by several workers (Beard and Hanson, 1992; Verma, 1994;

Jadhav and Siddique, 1999; Singh, 2008; Hadipour *et al.*, 2011; Khan *et al.*, 2011). The lungs were mostly congested, edematous and hemorrhagic which might be due to the degenerative and necrotic changes of the vascular endothelium caused by NDV. Haemorrhagic tracheitis with catarrhal exudates was also found present in many chickens in support of earlier findings (Alexander and Allan, 1974; Verma, 1994; Jadhav and Siddique, 1999; Singh, 2008). The gross lesions of the spleen and kidneys recorded during this study are almost same as described by Pazhanivel *et al.*, (2002), Nakamura *et al.* (2008) and Hadipour *et al.*, (2011) who recorded enlarged, congested and swollen kidneys with urate deposition. Pancreatic necrosis was also found in some birds, which is in agreement with Nakamura *et al.*, (2008) who reported the presence of white spots in pancreas. Most of the cases showed haemorrhagic enteritis with infiltration of mononuclear cells in the mucosa and sub-mucosa, while in some cases, there were congestion and necrosis of enterocytes of the intestines. Hemorrhages and necrotic changes were observed in the mucosa and sub-mucosa of proventriculus in many cases. Similar microscopic changes have also been described by many workers (Kindark *et al.*, 1996; Younus, 1996; Capua *et al.*, 2002; Pazhanivel *et al.*, 2002). The caecal tonsils showed hemorrhages, infiltration of heterophil in the lamina propria, lymphoid depletion and necrotic changes which support the previous reports (Stevens *et al.*, 1976; Capua *et al.*, 2002; Pazhanivel *et al.*, 2002). The microscopic changes of lungs and trachea observed during the present study are almost similar to those of Pazhanivel *et al.*, (2002) who found parabronchial lymphoid cell hyperplasia along with hypertrophy of bronchial epithelial cells, lymphocytic infiltration in lungs, loss of cilia in trachea, marked congestion and hemorrhage in alveoli. In most of the cases, the spleen sections showed depletion and necrosis of lymphocytes. Congestion, haemorrhages and interstitial nephritis observed in the kidney sections support the report of Nakamura *et al.*, (2008). Non-suppurative encephalitis and perivascular cuffing observed in the brain sections are in accordance with the earlier reports (Gowda and Eswaran, 1992; Kindark *et al.*, 1996; Nakamura *et al.*, 2008). In the present study, the clinical diagnosis was made on the basis of history, clinical signs, gross and microscopic lesions. Then, laboratory confirmation was done by RT-PCR for detection of the NDV viral genome (i.e. F gene). Out of 87 clinically ND suspected cases, 56 (64.37%) cases were found positive for NDV viral genome. Similarly, many workers had applied RT-PCR for detection of NDV (Schelling *et al.*, 1999; Hasan *et al.*, 2010; Gowthaman, 2011; Khan *et al.*, 2012; Thomazelli *et al.*, 2012; Madsen *et al.*, 2013; Mohammed *et al.*, 2013).

### Infectious bursal disease (IBD)

In the present study, the maximum cases of IBD was recorded in 3-6 weeks old birds (47.92%), which is in support of Lukert and Saif (1997) as well as the report of Mor *et al.* (2010) who found maximum cases (52.80%) in 21-30 days old birds followed by (33.13%) in 31-40 days old birds in Haryana. Similarly, Khan *et al.* (2009) also found that 4 weeks old broiler were highly susceptible to IBD (38%) followed by 3<sup>rd</sup> week (28%) and 5<sup>th</sup> week (28%) and no clinical case was found in first weeks of age. The younger chicks of 1-3 weeks, 6-9 weeks and 9-12 weeks old chickens were also found affected during the investigation, which is in conformity with the earlier reports of Fadley and Nazerian (1983) and Okoye and Uzoukwu (1981) respectively. The disease was found to occur

all around the year and the same was reported by Babiker *et al.* (2008). The percent morbidity varied from 3.5 - 5.4% while percent mortality varied between 38.5 - 52.6% during the period under study which is nearly similar to the previous reports (Kurade *et al.*, 2000; Saif *et al.*, 2000; Dey *et al.*, 2009). The low morbidity and mortality rates recorded during this present study might be due to regular vaccination of the chicks and proper managemental practices. The clinical signs like dullness, depression, anorexia, ruffled feathers and yellowish white or greenish yellow diarrhea recorded during the present investigation are in agreement with the findings of Islam and Samad (2004), Butcher and Miles (2012) and Rashid *et al.*, (2013). Most of the birds were disinclined to move and pecked at their vents and pericloacal feathers were stained with urates. Similar clinical signs had been reported by Cosgrove (1962) and Landgraf *et al.*, (1967).

The post-mortem findings of the present study included haemorrhages and darkened discoloration of thigh and breast muscles in most cases, which supports the reports of many researchers (Das *et al.*, 1981; Verma *et al.*, 1981; Lukert and Saif, 1997; Prabhakaran *et al.*, 1997; Islam and Samad, 2004; Singh, 2008; Sultana *et al.*, 2008). In most of the cases, bursa was congested, enlarged and swollen with accumulation of thick creamy or cheesy exudates, while in some cases; there were presence of gelatinous exudates around bursa. These lesions of the bursa are in agreement with the previous reports (Helmboldt and Garner, 1964; Cheville, 1967; Landgraf *et al.*, 1967; Skeeles *et al.*, 1979; Ley *et al.*, 1983; Younus, 1996; Zeleke *et al.*, 2005; Dutta *et al.*, 2007; Sultana *et al.*, 2008). The gross lesions of other organs like liver, spleen and proventriculus recorded during this present study are found almost similar to those described by Saif (2008), Morales and Boclair (1993), Prabhakaran *et al.*, (1997), Islam and Samad (2004) and Dutta *et al.*, (2007). In most of the cases, the kidneys were congested, enlarged and swollen, which might be due to deposition of urates caused by the enlarged bursa. Similar observations have been reported by Baxendale (2002), Cosgrove (1962), Younus (1996), Islam and Samad (2004) and Dutta *et al.*, (2007). Thymus in most cases was found to be enlarged, congested and haemorrhagic, which might be due involvement of virulent form of IBDV and secondary infections.

In present study, the bursa of Fabricius showed congestion, complete lymphoid depletion in the follicles leading to formation of cysts filled with necrotic debris, heterophils and hemorrhages in the interfollicular tissue. In few cases, areas of exudates, necrotic debris with severe heterophilic and lymphocytic infiltration in the bursal lumen were also recorded. These findings are in the line of earlier observations of several workers (Helmboldt and Garner, 1964; Cheville, 1967; Peters, 1967; Henry *et al.*, 1980; Okoye and Uzoukwu, 1981; Calnek *et al.*, 1992; Homer *et al.*, 1992; Yonus, 1996; Lukert and Saif, 1997; Zeleke *et al.*, 2005; Dutta *et al.*, 2007; Samanta *et al.*, 2008). Haemorrhages and necrotic changes with lymphoid depletion in caecal tonsils recorded during the present study period supports the findings of Uddin *et al.*, (2010) who observed significant reduction of lymphocytes in caecal tonsils, proventriculus, duodenum, jejunum, ileum and cecum. The spleen in most cases showed depletion of lymphocytes, congestion and focal or diffused areas of hemorrhage are in support of the previous reports (Helmboldt and Garner 1964; Dutta *et al.*, 2007). Congestion, degeneration of tubular epithelium degeneration and deposition of urates in tubular

lumen recorded during the present study are supported by the findings of Dutta *et al.*, (2007). Severe congestion in parabronchial area of lungs and microscopic changes of liver which showed congestion, degeneration of hepatocytes and lymphoid aggregations in portal areas might be due to involvement of virulent form of IBDV and secondary infections. The disease was clinically diagnosed on the basis of clinical history from the responsible persons of the farms, recorded clinical signs and gross and microscopic lesions of affected chickens. RT-PCR, a nucleic acid based detection test, was used as confirmatory diagnosis for the detection of IBD viral genome. Tissue samples comprising of bursa, spleen, thymus and liver from a total of 75 clinically IBD suspected cases were tested for detection of the F gene. Out of 75 IBD suspected cases, 48 (64%) cases were found positive. Similar diagnostic techniques have also been performed by several workers (Tham *et al.*, 1995; Jackwood and Jackwood, 1997; Kataria *et al.*, 1998; Muller *et al.*, 2003; Mittal *et al.*, 2005; Mahmood and Siddique, 2006; Zahoor *et al.*, 2010; Islam *et al.*, 2011; Barathidasa *et al.*, 2013). The present RT-PCR positive results (64%) is lower than that of Fatima *et al.*, (2013) who could detect 81 (95.29%) samples positive out of 85 bursal samples, which might be due to improper clinical diagnosis of IBD suspected cases.

### Fowl pox (FP)

During the present study period, the disease was observed to affect almost all age groups except the young chickens of 1-3 weeks old, as similarly observed by Jarmin (2006). It was found to occur all around the year as described by 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 clinical signs recorded during the study period included emaciation, anorexia, weakness and reluctance to move. Wart-like growths on the eyelid leading to impair vision were common findings. In some birds egg production was affected. Similar clinical signs have been described by Jordan *et al.*, (1996).

The common gross lesions observed in the present study were the wart-like nodular growths which were rough, large after coalescing, brown to grey colour on the face, eyelids and beaks. Similar lesions have also been described by some researchers (Yoshikawa *et al.*, 2002; Riper *et al.*, 2006). However, characteristic lesions for diphtheritic form of the disease were not observed in any of the affected birds. Similarly, 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 changes of the skin recorded during the present study included hydropic degeneration and hyperplastic epithelium of stratum spinosum which showed presence of eosinophilic inclusion bodies - Bollinger bodies. There was congestion & areas of necrosis in superficial layer of skin. These microscopic changes are in agreement with the findings of many workers (Reed and Schrader, 1989; Fallavena *et al.*, 1993; Gerlach *et al.*, 1998; Gortazar *et al.*, 2002; Smits *et al.*, 2003; Gulbahar *et al.*, 2005; Beytut and Haligur, 2007). A presumptive diagnosis was made by the presence of 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, PCR was also used

for confirming the disease by detecting the viral genome (i.e. 4b gene). Several researchers also used similar techniques (Oros *et al.*, 1997; Raue and Hess, 1998; Xie *et al.*, 1999; Ganesh, 2002; Rocke *et al.*, 2005; Kumar *et al.*, 2010; Asthana *et al.*, 2012; Thakor *et al.*, 2012; Susan *et al.*, 2014; Zheng *et al.*, 2015). Out of 21 fowl pox suspected cases, 5 cases (23.81%) were found positive for the viral genome. The present study reveals that some viral diseases namely; ND, IBD and Fowl pox are prevalent in poultry population in around Shillong, Meghalaya and the birds aged between 3-6 weeks are most vulnerable to these diseases which need immediate attention for prevention and control.

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