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RESEARCH ARTICLE

PATHOLOGY AND MOLECULAR DIAGNOSIS OF NEWCASTLE DISEASE IN CHICKENS AN AND AROUND 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. From 87 clinically suspected cases for ND, 56 cases were diagnosed as positive (15.14%) basing on the clinical history, gross and histopathology and finally confirmed by RT-PCR using a specific primer for F gene having a length of 1662 bp. Most cases of ND were found to occur in 6-9 weeks old birds (35.71%), followed by 3-6 (21.43%), 9-12 (19.64%), 1-3 (12.50%) and above 12 weeks old birds (10.71%) with percent morbidity and mortality of 35-50% and 25-35% respectively. Depression, emaciation, greenish or white diarrhea, torticollis, conjunctivitis, paralysis, drop in egg production and soft-shelled egg with respiratory signs were commonly observed. In few cases, chicken exhibited edema of the head, face and wattles. The changes in the proventriculus included pin point hemorrhages at the tips of the proventriculus glands, petechiae and ecchymoses in the mucosa. In both intestinal wall and caecal tonsils haemorrhagic ulcers were seen. Hemorrhagic tracheitis with congestion and catarrhal exudates were found in some cases. The spleens were enlarged, friable and dark red or mottled, while the kidneys were enlarged, congested and swollen with urate deposition. Significant microscopic changes were hemorrhages and necrosis of the proventriculus mucosa with mononuclear cell infiltration. Caecal tonsil revealed hemorrhages, infiltration of heterophils in the lamina propria, lymphoid depletion and formation of germinal centers. There were sloughing of tracheal mucosa with loss of cilia and congestion, congested and pneumonic lungs, while brain sections revealed non-suppurative encephalitis and perivascular cuffing. The present study suggests that Newcastle disease is occurring in chicken population in and around Shillong, Meghalaya.

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INTRODUCTION

Newcastle disease (ND) is an acute infectious viral disease of domestic poultry and other species of birds regardless of variation in sex and age (Haque *et al*., 2010). Chickens and guinea fowl appeared to be the most susceptible, followed by pheasants, turkeys and ostriches (Capua *et al*., 2000). The disease is considered endemic in wild birds worldwide and they act as a reservoir (Lindh *et al*., 2012). It causes huge economic losses to the commercial poultry farmers round the world (Diel *et al*., 2012).

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NDV is endemic in many countries of the world (Ashraf, 2014). The epizootics of ND in poultry continue to occur in Asia, Africa, Central and South America, while sporadic epizootics occur in Europe (Naveen *et al*., 2013). Major panzootics of ND had been recorded from different parts of the world (Qiu *et al*., 2011). The occurrence of ND has been reported to be more in the dry season i.e. in winter season (Nwanta *et al*., 2008). Virulent NDV causes 100% mortality in fully susceptible chicken; neurotropic velogenic form of NDV causes 100% morbidity and 50%-90% mortality in adult and young chicks, whereas in mesogenic and lentogenic mortality is usually low except in young chicks (Saif, 2008). The disease affects birds of all ages (Shankar, 2008).

Infection may take place by either inhalation or ingestion and spread from one bird to another depends on availability of the virus in an infectious form (Alexanderk, 1988). In acute and severe cases death is very sudden and birds die without showing any clinical signs (Calnek, 1991). In chickens, the general symptoms are loss of appetite, listlessness, abnormal thirst, weakness and drop in egg production, air sacculitis, tracheitis and conjunctivitis. Respiratory signs can include sneezing, gasping for air, nasal discharge and coughing, whereas a clear intestinal symptom is a greenish watery diarrhea. Nervous symptoms may consist of paralysis of wings or legs, twisting of head and neck or complete paralysis (Khan *et al*., 2011). The prominent gross lesions are haemorrhages in small intestines, glands of proventriculus and caecal tonsil (Hadipour *et al*., 2011).

Enlarged and congested spleen and swollen kidneys with urate deposition are recorded (Gowda, 1992). Mucosal haemorrhage and marked congestion of trachea, cloudy air sacs, caseous materials in trachea and bronchi are also common (Singh, 2008). Microscopically, catarrhal or hemorrhagic enteritis with infiltration of mononuclear cells in the mucosa and sub-mucosa, haemorrhages at tips of proventricular glands are common findings (Capua *et al*., 2000). There may be lymphocytic infiltration in lungs, loss of cilia in trachea, marked congestion and haemorrhage in alveoli (Pazhanivel *et al*., 2007). Caecal tonsil reveals infiltration of heterophils in the lamina propria, lymphoid depletion and formation of germinal centres (Nakamura *et al*., 2008). Brain shows disseminated non-suppurative encephalitis, neuronal degeneration, gliosis, focal spongiform changes and perivascular cuffing (Jadhav, 1999).

Clinical diagnosis based on history, signs and lesions may establish a strong index of suspicion but the laboratory confirmation is still required. Haemagglutination (HA) and Haemagglutination inhibition (HI) test, virus neutralization test, Enzyme linked immune-sorbent assay (ELISA), plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of the ND virus (Chaka *et al*., 2013). This study reports the prevalence of Newcastle disease in chickens in and around Shillong, Meghalaya and will be the basis for further studies for characterization of ND viruses. 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 and finally confirmed by detecting the viral genome with the help of reverse-transcriptase polymerase chain reaction (RT-PCR). In the present study, the disease was found to affect all age groups of birds as similarly described earlier (Shankar, 2008). Maximum cases of the disease were recorded in 6-9 weeks old chickens (30.71%), which support the findings of previous workers (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[10]. Some layer birds were seen to lay soft shelled eggs with severe drop in egg production, which had been similarly described by previous workers (Hadipour *et al*., 2011). Many birds were found dead suddenly with few or no symptoms, which supports the earlier description (Calnek, 1991). The most common gross changes recorded during the investigation were pin point hemorrhages at the tips of the proventricular glands (Fig.1), while in few cases there were congestion, petechiae and ecchymoses in the mucosa of the proventriculus.

Fig.1. Pin-point haemorrhages on tips of proventricular glands

Fig.2. Hemorrhagic lesions on caecal tonsils

Haemorrhagic necrotic ulcers and enteritis with exudates were also observed in the intestines, while haemorrhages and necrotic lesions in caecal tonsils (Fig.2) were also recorded in many cases. Similar lesions have been reported by several workers (Khan *et al*., 2011; Hadipour *et al*., 2011; Gowda, 1992).

Fig.3. Enlarged, friable and dark red or mottled spleen

Fig.4. Congested, edematous and hemorrhagic lungs

The gross lesions of the spleen and kidneys recorded during this study are almost same as described by the researchers (Hadipour, 2011; Nakamura *et al*., 2008**)** who recorded enlarged, congested and swollen kidneys with urate deposition. The lungs were mostly congested, edematous and hemorrhagic (Fig.4) 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 (Fig.5) are in support of earlier findings (Gowda,. 1992; Singh, 2008). In most of the birds, the spleens were enlarged, friable, mottled and dark red in colour (Fig.3). Pancreatic necrosis was also found in some birds, which is in agreement with the workers (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 (Fig.6). The caecal tonsils showed hemorrhages, infiltration of heterophils in the lamina propria, lymphoid depletion and necrotic changes.

Fig.5. Hemorrhagic tracheitis with congestion and catarrhal exudates

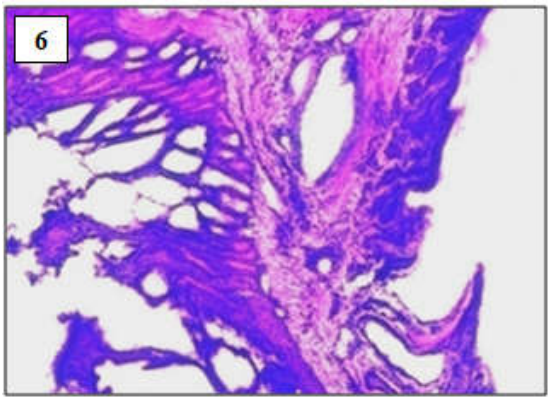

Fig.6. Proventriculus showing haemorrhages and mucosal necrosis (Hand E, 20X)

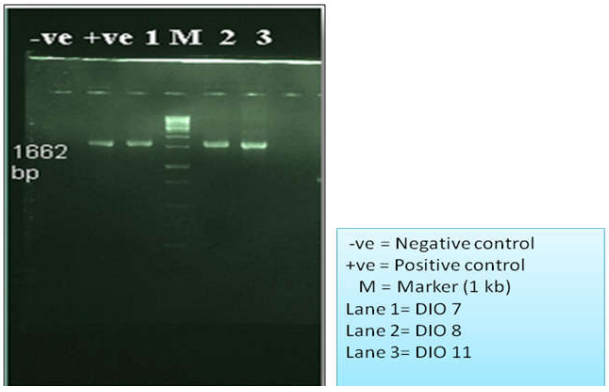

Fig. 7. 1.5% Agarose gel electrophoresis stained with Ethidium bromide showing the PCR products (1662 bp) of ND virus in tissue samples

Similar microscopic changes have also been described by many workers (Capua *et al*., 2008; Nakamura *et al*., 2008). The microscopic changes of lungs and trachea observed during the present study are almost similar to those of previous workers (Pazhanivel *et al*., 2002) who found para-bronchial 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 earlier report (Nakamura *et al*., 2008). Non-suppurative encephalitis and perivascular cuffing observed in the brain sections are in accordance with the earlier reports (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) (Fig.7). Out of 87 clinically ND suspected cases, 56 (64.37%) cases were found positive for NDV viral genome. Similarly, many workers (Thomazelli *et al*., 2012; Mohammed *et al*., 2013) had applied RT-PCR for detection of NDV. In conclusion, the present study revealed the prevalence of Newcastle disease (15.14%) in chicken in and around Shillong, Meghalaya which could be diagnosed basing on the clinical history, signs, gross and histopathology and confirmed by RT-PCR with detection of the viral genome.

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REFERENCES

Alexander, D.J. 1988. Newcastle disease: Methods of spread. In: Alexander ED (ed) Norwell, UK, Kluwer Academic, pp 256-272.

Ashraf, A, Shah, M.S. 2014. Newcastle Disease: Present status and future challenges for developing countries. *Academic journal,* 8(5): 411-416.

Bancroft, J.D., Stevens, A. 1980. Theory and practice of histological technique. Churchill Livingstone, New York, pp 89.

Calnek, B.W. 1991. Newcastle disease. In: Diseases of Poultry, Barnes HJ, Beard CW, Reid WM, Yoder HW (9th ed.). Iowa State University Press, Ames, Iowa-50010, pp 504-505.

Capua, I., Pozza, M.D., Mulinelli F, Marangon S, Terregino C. 2002. Newcastle disease outbreaks in Italy during 2000. *Vet Record,* 150: 565-568.

Chaka H, Goutard F, Gil P, Abolnik C, Almeida R, Bisschop SPR, Thompson PN. 2013. Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. *Trop Anim Health Prod,* 45: 705-714.

Diel DJ, Susta L, Garcia SC, Killian ML, Brown CC, Miller PJ, Afonso CL. 2012. Complete genome and clinicopathological characterization of a virulent newcastle disease virus isolate from South America. *J Clin Microbiol,* 50(2): 378-387.

Gowda RNS, Eswaran R. 1992. Ranikhet disease in pigeons: Clinicopathological studies. *Indian J Vet Patient*, 16(2): 95-97.

Hadipour MM, Habibi GH, Golchin P, Hadipourfard MR, Shayanpour N. 2011. The Role of Avian Influenza, Newcastle Disease and Infectious Bronchitis Viruses during the Respiratory Disease Outbreak in Commercial Broiler Farms of Iran. *International J Anim Vet Advances,* 3(2): 69-72.

Haque MH, Hossain MT, Islam MT, Zinnah MA, Khan MSR, Islam MA. 2010. Isolation and detection of Newcastle disease virus from field outbreak in broiler and layer chickens by reverse transcription- polymerase chain reaction. *Bangl J Vet Med.* 8 (2): 87–92.

Jadhav NV, Siddique MF. 1999. Handbook of Poultry production and management. Printed edn. Jaypee Brothers, Medical Publishers, New Delhi, pp 1990.

Khan MY, Arshad M, Mahmood MS, Hussain I. 2011. Epidemiology of Newcastle disease in rural poultry in Faisalabad, Pakistan. *Int J Agric Biol*, 13: 491–497.

Lindh E, Ek-Kommonen C, Vaananen VM, Alasaari J, Vaheri A, Vapalahti O, Huovilainenb A. 2012. Molecular Epidemiology of Outbreak-Associated and Wild-Waterfowl-Derived Newcastle Disease Virus Strains in Finland, Including a Novel Class I Genotype. *J Clinical Microbiology,* 50(11): 3664–3673.

Mohammed MH, Zahid AAH, Kadhim LI and Hasoon MF. 2013. Conventional and molecular detection of Newcastle disease and infectious bursal disease in chickens. *J. World's Poult. Res.,* 3:05-12.

Nakamura K, Ohtsu N, Nakamura T, Yamamoto Y, Yamada M, Mase M, Imai K. 2008. Pathologic and immunohistochemical studies of Newcastle disease (ND) in broiler chickens vaccinated with ND: severe non-purulent encephalitis and necrotizing pancreatitis. *Vet. Pathol,* 45(6): 928-933.

Naveen KA, Singh SD, Kataria JM, Barathidasan R, Dhama K. 2013. Detection and differentiation of pigeon paramyxovirus serotype-1 (PPMV-1) isolates by RT-PCR and restriction enzyme analysis. *Trop Anim Health Prod,* 10: 01-06.

Nwanta JA, Egege SC, Alli- Balogun JK, Ezema WS. 2008. Evaluation of prevalence and seasonality of Newcastle disease in chicken in Kaduna, Nigeria. *World's Poultry Sci J*, 64(03): 416-423.

Olabode HOK, Damina MS, Ahmed AS, HMED, Moses GD and Wungak YS. 2012. Retrospective study of Newcastle disease in commercial poultry farms in Ilorin, Kwara State-Nigeria. *Vom J. Vet. Sci.,* 9: 66 -70.

Pazhanivel N, Balasubramaniam GA Manohar MB. 2002. Pathology of New castle disease virus infection in immense and non-immense chicken. *Indian Vet J,* 79: 29-32.

Qiu X, Sun Q, Wu S, Dong L, Hu S, Meng C, Wu Y, Liu X. 2011. Entire genome sequence analysis of genotype IX Newcastle disease viruses reveals their early-genotype phylogenetic position and recent-genotype genome size. *Virol J*, 8: 01-11.

Saif YM. 2008. Newcastle disease, other Avian Paramyxoviruses and Pneumovirus infection, Infectious bronchitis, Infectious bursal disease, Laryngotracheitis, leukosis/Sarcoma Group. In: Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE(ed) Diseases of Poultry, 12th edn. Blackwell Publishing State Avenue, Ames, Iowa, USA pp 82-88, 126-130, 191-198,138-146, 528-535.

Shankar BP. 2008. Common Respiratory Diseases of Poultry: review. *Vet World,* 1(7): 217-219.

Singh KS. 2008. Studies on some common diseases of birds with special reference to Infectious bursal disease, Ranikhet disease, Colibacillosis and Gangrenous dermatitis. Ph.D. Thesis submitted to West Bengal University of Animal and Fishery Science, 37, 38, KB Sarani, Kolkata 700037.

Thomazelli LM, Araujo J de, Ferreira C, de S, Hurtado R, Oliveira DB, Ometto T, Golono M, Sanfilippo L, Demetrio C, Figueiredo ML and Durigon EL. 2012. Molecular surveillance of the Newcastle disease virus in domestic and wild birds on the North Eastern Coast and Amazon Biome of Brazil. *Brazilian J. Poult. Sci.,* 14:01-07.

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