Indian J. Vet. Pathol., 35(2) : 168-170, 2011

Pathology and molecular diagnosis of Newcastle disease virus infection in broiler breeders

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Received: 25.11.2011; Accepted: 27.12.2011

ABSTRACT

Gowthaman, V., Singh, S.D., Dhama, K., Barathidasan, R., Anjaneya and Ramakrishnan, M.A. (2011). Pathology and molecular diagnosis of Newcastle disease virus infection in broiler breeders. Indian J. Vet. Pathol., 35(2) : 168-170.

Newcastle disease (ND) is one of the most devastating diseases of poultry causes a fatal respiratory, enteric and neurological disease leading to almost 100% morbidity and mortality. Although, Newcastle disease virus (NDV) infections reported in many avian species and different types of chickens viz. broilers and layers, reports on NDV infections in breeders are scanty. In the current study, disease investigation was carried out in a 35-weeks-old broiler breeder flock with heavy mortality and severe respiratory disease in Bareilly district of Uttar Pradesh. Necropsy was carried out on freshly dead and ailing birds. In the virus isolation study, embryonating chicken eggs were died within 48 hours of inoculation and showed HA titer of 2^9. The NDV was confirmed by F gene based RT-PCR from amnio allantoic fluids samples. The present study confirms the existence of NDV infections in the breeders and emphasizes the need for developing effective vaccine programme and biosecurity measures to combat the Newcastle disease.

Keywords: Broiler, haemagglutination assay, Newcastle disease, pathology, RT-PCR

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INTRODUCTION

Newcastle disease (ND) is one of the most devastating diseases of poultry causes a fatal respiratory, enteric and neurological disease leading to 100% morbidity and mortality. The etiological agent is Newcastle disease virus (NDV), also known as avian paramyxovirus type-1 (APMV-1) - a member of the genus *Avulavirus* of the subfamily *Paramyxovirinae* in the *Paramyxoviridae* family under the order *Mononegavirales*[1]. It is a single stranded, non-segmented, enveloped RNA virus with negative polarity. The NDV has ~15 kb RNA genome composed of six genes that codes for six corresponding viral proteins[2]. Although NDV infect over 250 avian species but clinical entity is most important in domestic chickens[3]. Clinical signs are highly variable and depend on the nature of the infecting virus, dose and the degree of immunity from previous exposure or vaccination. Based on the severity of the disease in chickens, NDV has been classified into three pathotypes: lentogenic, mesogenic and velogenic. Lentogenic strains cause subclinical infection with mild respiratory or enteric disease and are considered low-virulent. Mesogenic strains are of intermediate virulence causing respiratory infection with moderate mortality (< 10%), while velogenic strains are highly virulent causing mortality rates up to 100%. Velogenic strains are further classified into viscerotropic velogenic and neurotropic velogenic strains. Viscerotropic velogenic strains produce lethal haemorrhagic lesions in the viscera, whereas neurotropic velogenic strains cause neurological and respiratory disorders[4]. The NDV outbreaks regularly occur in commercial and village chicken and rarely reported in breeders[5]. This paper describes the pathology and molecular diagnosis of NDV infections in broiler breeders.

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MATERIALS AND METHODS

Disease investigation was carried out in a 35-weeks-old broiler breeder farm having heavy mortality (>80%) and severe respiratory disease in Bareilly district of Uttar Pradesh. The flock housed with multi aged breeder and maintained under deep litter system. The flock had no history of vaccination for past six months. Necropsy was carried out on freshly dead and ailing birds. Tissue samples such as trachea, lungs, air sacs, liver, were collected aseptically for virus isolation and molecular diagnosis. Small pieces approx. 3-5 mm size from the visceral organs were preserved in 10% buffered formol saline for histopathology. The samples were properly stored at -20°C for PCR and at room temperature for histopathological studies. The tissue samples were processed using paraffin embedding technique to get 5µ thick sections and stained with haematoxylin and eosin technique. For the virus isolation, samples were processed and inoculated in to 9-11days-old embryonating chickens through allantoic route. For identification of NDV, haemagglutination and RT-PCR assays were used. HA was carried out from the harvested amnio allantoic fluid (AAF) as per OIE 2009 (http://www.oie.int/fileadmin/ Home/eng/Health_standards/tahm/2.03.14_NEWCASTLE_DIS.pdf). HA positive samples were further subjected to F gene based RT-PCR test for molecular confirmation of NDV[6].

RESULTS

Clinical signs

The spread of the disease was rapid and the mortality rate was very high (>80%). The general clinical signs consisted of depression, severe prostration, somnolascence, reduction in normal vocalizations, and decrease in food and water consumption, huddling behavior, ruffled feathers and greenish diarrhea. A wide range of consistent and progressive neurological signs including tremors of head and neck, inability to stand, torticollis, paresis, paralysis, convulsions, rolling or circling movements, incoordination, loss of balance and recumbency with pedaling movement, flapping movements of the wings, and unusual positions of head and appendages could be noticed in the affected flock. Birds exhibited respiratory signs viz. labored breathing, increased rales, wheezing, and open-mouthed breathing and enteric signs viz. watery/tenacious mucus discharge from the nostrils.

Gross and histopathology

Mucosal surface of the trachea was stripped with numerous red streaks of congestion/haemorrhage and the lumen contained catarrhal exudates. The lungs exhibited severe and diffuse bilateral pneumonia and pleurisy. Multiple petechiae were seen in the epicardium, surface of the proventriculus fat and serosal surface of peritoneum. Many birds exhibited polyserositis with pericarditis, perihepatitis, and/or egg peritonitis. The air sacs were opaque, thickened and exhibited increased vascularity (Fig. 1). Marked to severe, acute multifocal haemorrhages and accumulation of tenacious mucus could be observed on the tips of the proventriculus glands. The intestine was devoid of feed and contained tenacious mucous. The spleen was severely atrophied, and peritoneal cavity contained fibrinous/cheesy yolk/purulent material and accumulation of milky white to yellowish white fluid due to ruptured ovarian follicles as a consequence of chronic egg yolk peritonitis (Fig. 2).

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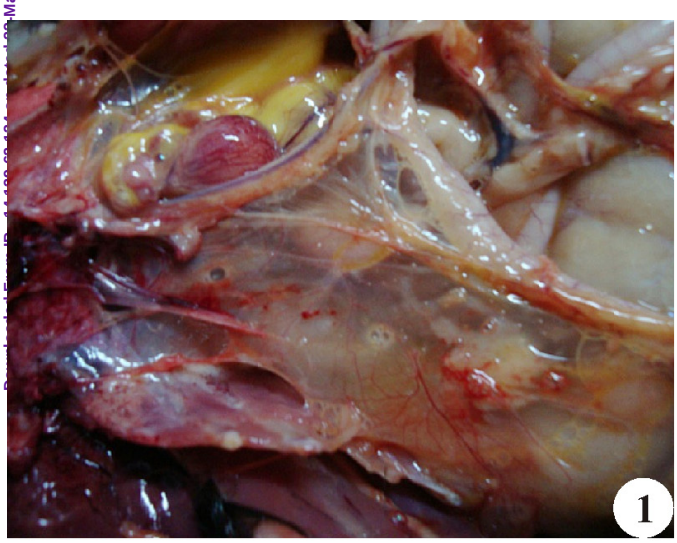

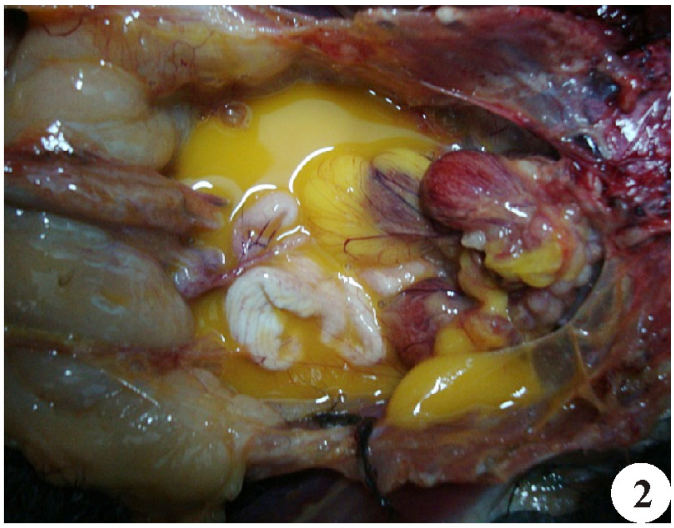

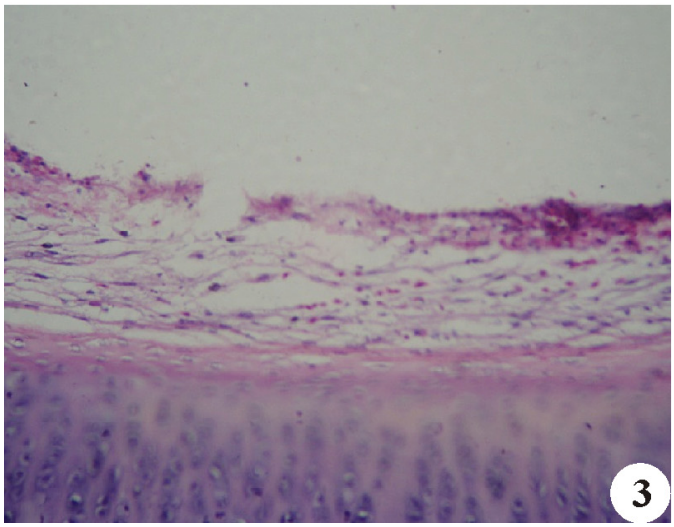

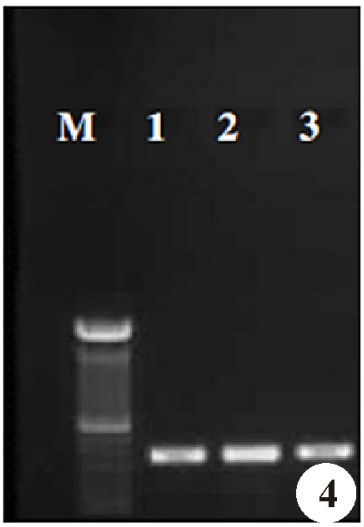

Fig. 1. Thickened and cloudy airsacs. **Fig. 2.** Peritoneal cavity contains yellowish yolk fluid due to rupture of ovarian follicles. **Fig. 3.** Tracheal epithelium showing necrosis and the lamina propria was expanded with inflammatory cells and exudate. H&E x200. **Fig. 4.** NDV-specific amplicons visualized by agarose gel electrophoresis. Lane M, molecular weight marker; lanes 1 to 3, samples; Lanes 1, 2, 3 show the NDV-speciûc 356 base pairs (bp) product.

Renomegaly with pallor parenchyma and accentuated lobular surface architecture could be noticed in most of the birds. The ovary contained follicles of various stages of development and many of them were atretic.

Diffused and severe microvascular engorgement could be noticed in the comb and wattles. The lung was one of the most severely affected organs. Severe necrosis and desquamation of the epithelial lining with consequent exposure and rupture of capillaries led to massive haemorrhage in tracheal lumen (Fig. 3). The lesions consisted of vascular engorgement, congestion and haemorrhages in the bronchial submucosa, air and blood capillaries, smooth muscle hypertrophy in tertiary bronchus, intense peribronchial infiltration of mononuclear cells, epithelial thickening, and accumulation of fibrinous material in bronchi. Severe haemorrhages within the mucosal ridges of the proventriculus was suggestive of haemorrhagic proventriculitis. Lesions in the alimentary tract were largely confined to the apical epithelial villi, crypts, and blood vessels. Degeneration and sloughing of epithelial cells covering tips of villi, and mononuclear infiltrations were suggestive of mucous enteritis. The spleenic lesions viz. focal vacuolation and destruction of lymphocytes in the follicular areas were suggestive of immunosuppression. In kidneys, tubular degeneration and necrosis was associated with mild interstitial haemorrhage.

The embryos died within 48 hours post inoculation and HA titer of 2^9 could be observed from AAF. AAF was screened for F gene based RT-PCR and NDV nucleic acid (amplicon size 356 bp) could be detected from all the AAF (Fig. 4).

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DISCUSSION

In the present study, disease investigation was carried out in a broiler breeder flock with heavy mortality and severe respiratory disease. NDV could be isolated from the affected flocks by embryonating chicken eggs inoculation and identified by HA and F gene based RT-PCR methods. The birds exhibited general depression, dullness, somnolascence, loss of appetite, respiratory distress, greenish diarrhoea, and neurological disease. Similar findings were also recorded in broiler flocks in Jordan by Roussan *et al*.[7,8]. The gross pathological changes including haemorrhagic/catarrhal tracheitis, purulent casts in trachea, pulmonary congestion and oedema, abdominal airsacculitis, fibrinous perihepatitis, petechiae in serosal layer of heart and abdominal fat, haemorrhagic proventriculitis, catarrhal enteritis, necrotic pancreatitis, atrophy of spleen, egg peritonitis, oophoritis, nephritis-nephrosis complex and cloacitis were in agreement with the earlier descriptions of ND[9-11]. The histological alterations observed like hyperplasia of surface epithelium with loss of cilia in the trachea, congestion, oedema, moderate cellular infiltration of lymphocytes and macrophages in early stages of the disease and diffuse marked destruction of epithelium along with severe submucosal oedema in advanced stage observed in this study were in agreement with the earlier reports[12,13]. NDV can transmit vertically[5,14]. Capua *et al*.[5] reported the isolation of velogenic NDV from breeder hen, embryonated eggs and progeny chicks from a flock following the episode of mortality. The current study emphasizes the need for developing an effective vaccine programme and strict biosecurity measures to combat the NDV infections in breeders.

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