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Gross and histopathological identification of Newcastle disease virus (NDV) in broiler chickens from Bangalore, Karnataka

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Abstract
Newcastle disease (ND) is an economically important and highly transmissible viral disease that affects many avian species, including domestic poultry. Samples were collected from different backyard broiler farms in Bangalore, Karnataka. Further, samples were subjected to gross examination and histopathological examination to detect the presence of Newcastle disease in the field samples. Gross examination reveals the typical Newcastle disease virus (NDV) lesions and histopathological examination revealed severe haemorrhages and congestion in the organs.

Keywords: Newcastle disease virus, gross examination, histopathological examination

Introduction
Newcastle disease (ND) is an acute contagious disease affecting most of the avian species. It causes heavy morbidity and mortality in the poultry industry. ND is a fatal disease in poultry that is notifiable to the World Organization for Animal Health (OIE). ND is caused by virulent forms of Avian Paramyxovirus 1 (APMV1). The Newcastle disease virus belongs to the genus Avian Aulavirus of the subfamily *Avulavirinae*, family *Paramyxoviridae* and order *Mononegavirales* (Amarasinghe *et al.*, 2019) [3]. NDV has a wide host range including wild birds approximately 241 species of 27 orders, out of known 50 orders of birds (Madadger *et al.*, 2013) [13].
The NDV is an enveloped, single stranded negative sense RNA virus whose genome is approximately 15kb. The genome has 6 open reading frames (ORFs) which encode for 6 major structural proteins, namely, nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and the RNA-dependent RNA polymerase (L) (Ganar *et al.*, 2014) [20]. HN protein is responsible for inducing haemagglutination of red blood cells and binding to the host cell receptors. HN protein activates the Fusion protein, which is responsible for the fusion of the viral envelope to the host cell membrane (Acheson *et al.*, 2011) [1]. High molecular variability and association with virulence, the F gene has been widely used as a target for the genotype classification of NDV (Diel *et al.*, 2012; Dimitrov *et al.*, 2019) [7, 8]. Two major groups are classified i.e. class I and class II viruses, based on the complete sequence of the F gene. Class I belongs to a single genotype, whereas the more genetically diverse class II viruses are currently divided into more than 20 distinct genotypes separated by nucleotide distances above 10% (Dimitrov *et al.*, 2019) [8]. NDV cause very severe infection with virulent strains. Clinical signs can range from respiratory distress to neurologic signs and systemic illness, and mortality can reach 100%. NDV strains are classified based on the virulence into Velogenic, mesogenic, lentogenic and avirulent respectively (Suarez *et al.*, 2020) [17]. NDV outbreaks occur worldwide and endemic in many countries in Asia and Africa (Balachandran *et al*., 2014; Suarez *et al*., 2020; WAHIS, 2019) [4, 17, 19]. Though endemic reports are available in India, information about NDV in Karnataka state is still limited. Newcastle disease outbreaks were studied from an unorganised broiler farm in the Bengaluru district, Karnataka.

Materials and Methods
Sample collection
Samples were collected from backyard broiler chicken farms located in the Bengaluru district

Of Karnataka state during the year 2022. Cloacal swabs from live birds and pooled tissue samples such as intestines, spleen and lungs from dead birds were collected. Swab samples were collected in a virus transport medium with antibiotics. Samples were placed immediately in ice packs and transported to the laboratory and stored at -80 °C until further processing.

Gross pathology

The dead birds were subjected to gross examination and suitable samples were collected from post mortem examination. Pathological changes in different organs were recorded in detail. Tissues from the lungs, proventriculus and bursa of Fabricius of NDV infected dead chickens were collected aseptically in sterile containers for virus detection. Newcastle disease virus lesions were documented during the postmortem examination.

Histopathological examination

Proventriculus from infected chickens was collected in 10% neutral buffered formalin for histopathological examination. The tissue samples were processed by using the paraffin embedding technique for the preparation of sections and stained with haematoxylin and eosin (H&E) for observing microscopic changes caused by NDV (Suvarna *et al.*, 2019) [18].

Results

Gross lesions

The dead birds were examined for gross pathological changes during postmortem examination. Severe congestion, hemorrhages and consolidation of the lungs were observed. The proventriculus of infected chickens showed hemorrhages on the tip of the proventricular papillae (Fig. 1a and 1b). Multifocal button-like ulcers of various sizes with raised margins were noticed in the intestinal mucosa. Cecal tonsils were enlarged and hemorrhagic (Fig. 1c). Other visceral organs such as the liver and kidneys showed mild congestion and hemorrhages. Spleen was atrophied with multifocal necrotic foci. Bursa of Fabricius was edematous and contained yellowish caseous material in the lumen.

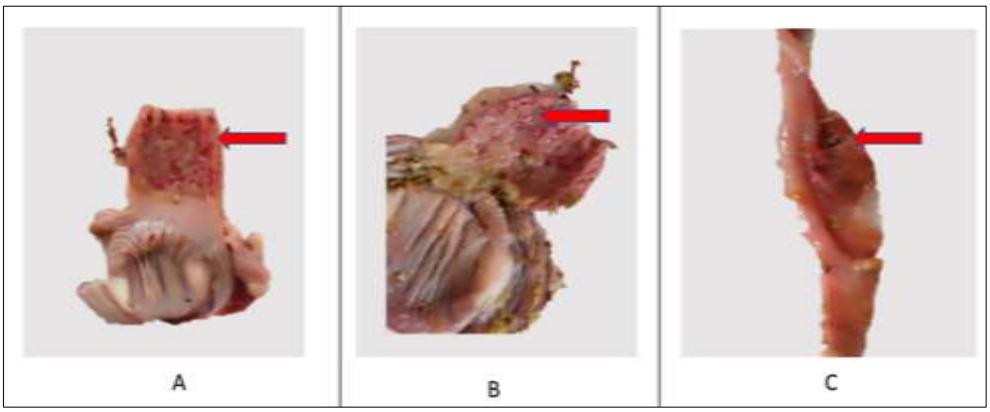
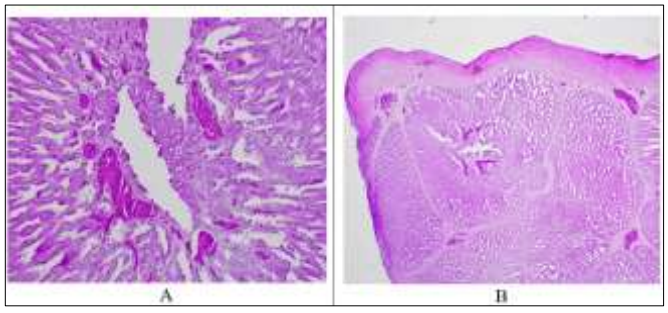


Fig 1: Gross pathological lesions in the proventriculus and cecal tonsils of chickens infected with NDV

Histopathology

The proventriculus showed severe congestion and hemorrhages in the finger-like mucosal folds (plicae), edema, loss of lining epithelium, fusion and shortening of plicae and mild infiltration of inflammatory cells.



A) Sections of the proventriculus of infected chickens showing severe congestion and haemorrhage 10x Proventriculus
B) Epithelial hypertrophy and haemorrhage 4x Proventriculus.

Fig 2: Histopathological changes in the proventriculus of NDV infected chickens.

Discussion

The chronological events of the NDV outbreak leading up to death were severe lethargy exhibited by these birds in addition to anorexia and greenish diarrheic faeces. Strains of NDV are highly variable in their pathogenicity for chickens and amino acid residues at the fusion protein cleavage site (FPCS) are postulated as the primary determinant of NDV virulence (OIE, 2018; Samal *et al.*, 2011) [16]. The HN protein with receptor recognition and neuraminidase activities of the virus determines and contributes to the virulence of NDV (Huang *et al.*, 2004; De Leeuw *et al.*, 2005) [10, 6]. Virulent strains of NDV are defined by the World Organization for Animal Health (OIE) as viruses that have an intracerebral pathogenicity index (ICPI) of 0.7 or higher (2.0 is maximum) or a fusion protein cleavage site (FPCS) with multiple basic amino acids and phenylalanine at position 117 (OIE, 2018). The onset of clinical signs in NDV infected chickens could vary depending on the age and genetic endowment of the birds and dose and route of infection, genotype, host adaptability and pathogenicity of the virus (Alexander, 2000). The predominant post-mortem lesions observed in the present study include petechial haemorrhage on the tip of the proventricular papilla and caecal tonsils, hemorrhages and consolidation of lungs and multiple button like ulcers in the intestinal mucosa. The gross lesions observed in this study were suggestive of ND. The most common ND lesions reported were matting of vent feathers, petechiae haemorrhage on the tip of the proventriculus papilla, ventriculus, intestine, and cecal tonsil, tracheal and lung congestion, and ovarian follicle degeneration (Balachandran *et al.*, 2014) [4]. The clinical signs of a highly virulent NDV infection in chickens can be divided into 2 pathotypes;

Virulent strains that cause diarrhoea and frequent hemorrhagic intestinal lesions are called viscerotropic velogenic NDVs. On the other hand, strains that cause respiratory and neurotropic signs are called neurotropic velogenic NDVs (Alexander and Allan, 1974; Balachandran *et al.*, 2014) [2, 4].

Based on observation of clinical symptoms in digestive organs, it is known that the ND infection was of velogenic type. NDV velogenic type infection can cause more severe infection compared to mesogenic or lentogenic NDV. Mesogenic NDV infection can cause macroscopic and microscopic lesions; however, the lesions will spread as much as velogenic virus infection (Kommers *et al.*, 2001) [12]. NDV replication within intestinal lymphoid follicles causes hemorrhage and edema in internal organs due to blood vessel injury (Eze *et al*., 2014) [9]. That field isolate of NDV antigen was found immunopositive in the pancreas, duodenum, proventriculus, and Bursa Fabricius (Nakamura *et al.*, 2008; Bwala *et al*., 2012) [14, 5].

The high incidence of ND in vaccinated and unvaccinated chicken in this research showed that vaccination program currently conducted in the field is not protective enough in preventing disease infection. At present, ND vaccination only protects poultry by decreasing the severity of the disease and lowering mortality, however, it cannot prevent NDV replication, especially virulent ND (Kapczynski *et al.*, 2013; Balachandran *et al.*, 2014) [11, 4].

Conclusion

ND is a major threat to the poultry industry around the globe. The present outbreaks of NDV infection in broiler farms are due to the velogenic nature of the virus. Further, molecular characterization is essential to determining the genotype of velogenic ND that is causing these outbreaks. However, as evident from the multiple outbreaks occurring, current vaccination strategies are not fully efficacious, and the development of new concepts for vaccine generation is needed.

Conflict of interest

The authors declare no conflict of interest.

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