



## **Review on economic importance and current diagnostic techniques of Newcastle disease in poultry**

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### **Abstract**

Newcastle disease remains a constant threat to the poultry industry and is a limiting disease for poultry producers worldwide. Newcastle disease is a contagious bird disease affecting many domestic and wild avian species; it is transmissible to humans. Newcastle disease is an important infectious disease of the poultry that is caused by virulent strains of Avian Paramyxovirus -1, which is a single strand non segmented negative sense RNA virus. Newcastle disease is an economically important disease and also a major threat to poultry industry. According to variation in strains of NDV, the rate of mortality and morbidity in a flock is variable. Clinical diagnosis based on history, signs and lesions in addition hemagglutination and hemagglutination inhibition test, virus neutralization test, Enzyme linked immune-sorbent assay, plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of the ND virus. The transmission of NDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing. Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhoea, depression and in appetite, partial or complete drop in egg production and an increased production of deformed eggs. Gross and microscopic lesions as with clinical signs, the organs affected in birds infected with NDV are dependent on the strain and pathotype of the infecting virus, in addition to the host and all the other factors that may affect the severity of the disease. The objective of this review is to understand the economic importance and current diagnostic techniques of Newcastle disease in poultry.

**Keywords:** Economic important, Diagnosis, New castle disease, Poultry

### **Introduction**

Poultry, the largest livestock group, account for more than 30% of all animal protein. However, this production is mainly based on commercial poultry, which accounts for only 20% of the total poultry population (Pemin *et al.*, 2001). Poultry represents an important sector in animal production, with backyard flocks representing a huge majority, especially in the developing countries. In these countries, villagers raise poultry to meet household food demands and as

additional sources of incomes. Newcastle disease is a contagious bird disease affecting many domestic and wild avian species; it is transmissible to humans (Nelson *et al.*, 1952). Newcastle disease (ND) is a viral disease of poultry caused by a single-strand, nonsegmented, negative-sense RNA virus known as Avian paramyxovirus 1 (APMV-1). Availability of egg is increasing at rate of round about 4% annually, White meat's essential nutrients are same as red meat, but white meat has the advantage of containing less cholesterol and saturated fat.

In most developing countries, meat is a very important protein sources in diet of people because it is affordability and has high quality protein (Thomazelli *et al.*, 2012). In developing countries; the broiler meat is the cheapest source of animal protein. In developing countries, where the majority of chickens are reared under “backyard” subsistence conditions, ND can drastically limit the amount of dietary protein as well as damage the microeconomy due to loss of ability to sell off extra chickens or eggs. Where chickens are raised commercially, either in developing or developed countries, outbreaks have occurred in many locations, causing massive economic damage through control efforts and trade losses. For instance, during the last major outbreak in the United States, in California in 2002–2003, more than 2,500 premises were depopulated (4 million birds) at a cost of US\$162 million. Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhoea, depression and in appetence, partial or complete drop in egg production and an increased production of deformed eggs (Kahn CM, 2005).

According to variation in strains of NDV, the rate of mortality and morbidity in a flock is variable. Pathotyping of Newcastle disease viruses by RT-PCR and restriction enzyme analysis along with decrease in egg production (Choi KS, *et al.*, 2010). Isolation of virus and serological diagnostics, such as HI Test, ELISA and molecular diagnostic tests like real time PCR confirmed the presence of velogenic Newcastle Disease Virus. The objective of this review is to understand the Newcastle disease causative agent, pathogenicity, clinical sign and how to prevent and control the Newcastle disease, which concerned with the currently published or reported research. Recently, the disease which decreases the development of poultry production for industry is the infectious diseases, among infection disease Newcastle is the one which causes economical lose of poultry and its product.

Objectives:-

- To understand the economic importance Newcastle disease in poultry
- To review the current diagnostic techniques of Newcastle disease

## Literature Review

### Etiology

All avian paramyxoviruses (APMV) are part of the genus Avulavirus, subfamily Paramyxovirinae, family Paramyxoviridae, order Mononegavirales. There are 9 serotypes of APMV, but all isolates of Newcastle disease virus (NDV) belong to serotype 1 (APMV-1), therefore NDV is synonymous with APMV-1(Lamb *et al.*, 2005).

### Occurrence and Economic Important

Three categories of viral pathogenicity result in different clinical forms of the disease. Velogenic-viscerotropic virus (vvND) infection results in acute onset, highly lethal disease. Mesogenic virus causes acute, moderately lethal disease with nervous and respiratory signs. Lentogenic virus is responsible for mild respiratory infection. Velogenic and mesogenic forms are exotic to the USA, Canada, the UK and other European countries but are widespread in Asia, Africa, and Latin America. The lentogenic form is encountered in most poultry-producing areas including the USA. Severe losses from mortality, depressed egg production and lowered feed conversion efficiency occur as a result of exposure to vvND. The lentogenic form is responsible for erosive losses in broilers including lowered gain and feed conversion efficiency and elevated mortality and condemnation. The severity and financial impact depends on climatic and management stress and inter current exposure to pathogenic E. coli and other viral respiratory disease and immunosuppressive agents. The cost and consequences (respiratory stress) of vaccination are significant, especially during winter and following immunosuppression. Disruption of trade and the cost of eradication of vvND in non-endemic countries impose a significant burden on producers and the public sector after outbreaks (Chang and Dutch , 2012).

Virulent NDV strains are endemic in poultry in most of Asia, Africa, and some countries of North and South America Other countries, including the USA and Canada, are free of those strains in poultry and maintain that status with import restrictions and eradication by destroying infected poultry. Cormorants, pigeons, and imported psittacine species are more commonly infected with vNDV and have also been sources of vNDV infections of poultry. NDV strains of low virulence are prevalent in poultry

and wild birds, especially waterfowl. Infection of domestic poultry with lNDV contributes to lower productivity (Merck, 1995).

ND virus is infective for almost all avian species, both domestic and wild. Chickens are highly susceptible to infection with Newcastle disease virus, including the pigeon variant of APMV-1. Considered to be the most susceptible of domestic poultry species. Newcastle disease virus is heat stable when compared with most of paramyxovirus. It remains infectious in bone marrow and muscle of slaughtered chicken at least six months at -20°C and for up to four months in refrigerator temperature and also infectious virus may survive for months at room temperature in eggs laid by infected hens and for over a year at 4°C. Higher prevalence of ND is during dry season than wet season. However, rare higher prevalence of ND is also seen during wet season that may be related to Ethiopian Holidays (Filseta, Enkutatash etc) celebrated during wet season. Human activity and increased turnover in the chicken markets during dry season could lead to outbreaks of NCD that have been attributed to high prevalence during dry season (Nega *et al.*, 2012).

As studies reported on Newcastle disease that indicated high significant difference in NCD prevalence between local and cross breeds of chickens. Highest prevalence's are recorded in cross breeds of chickens than local breed [39]. The low altitudes do have higher prevalence than the mid and high (Belayeh *et al.*, 2014). Mortality may be very high, often reaching 50 to 100%. The prevalence of NCD varies among years in Ethiopia.

### Transmission

Newcastle virus is highly contagious. Infection occurs either by the inhalation of virus in aerosol form or ingestion of contaminated feed or litter. Wind dispersal may occur over distances of 5 km. The transmission of NDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing (Desalegn JM, 2015). Newcastle disease is very contagious and is easily spread from one bird to another. The infection is usually transmitted by direct contact with sick birds or unaffected birds carrying the virus. Even vaccinated birds that are clinically healthy can excrete virulent virus after they have been exposed. Virus can also be transmitted indirectly by

people, other animals, equipment, vehicles, contaminated poultry products, feed and water (Serkalem *et al.*, 2005).

During the course of infection of most birds with NDV, large amounts of virus are excreted in the feces. Ingestion of feces results in infection; this is likely to be the main method of bird-to-bird spread for avirulent enteric NDV and the pigeon variant virus, neither of which normally produces respiratory signs in infected birds (Caupa and Alexander, 2009). Vertical transmission (i.e., passing of virus from parent to progeny via the embryo) remains controversial. The true significance of such transmission in epizootics of ND is not clear. Experimental assessment using virulent viruses is usually hampered by cessation of egg laying in infected birds. Infected embryos have been reported during naturally occurring infections of laying hens with virulent virus (Beard and Hanson, 1984), but this generally results in the death of the infected embryo during incubation.

### Clinical Signs

Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhoea, depression and inappetence, partial or complete drop in egg production and an increased production of deformed eggs (Kahn CM, 2005). Clinical sign and course of disease can be grouped into four different pathotypes based on the strains of Newcastle disease virus (Cole and Hutt, 1961). These all four pathotypes are listed as follows:

### Viscerotropic velogenic Newcastle disease

This form is characterized by acute onset with up to 100% flock morbidity and rapidly ascending high mortality (20% in 2 days, 50% in 3 days, 80% in 5 days) accompanied by respiratory and nervous signs. In susceptible commercial egg production flocks and breeders, peracute cessation of production occurs with the presence of shell-less eggs due to premature oviposition. Exposure of immunized flocks results in variable decline in production. That can be seen are obvious depression, inappetence, substantial drop in egg production, increased respiration, a profuse greenish-yellow diarrhoea that rapidly leads to dehydration and collapse, swollen heads and cyanotic

combs. Mortality can be up to 90% and infected birds usually die within one or two days. Birds that survive the initial phase often develop nervous signs. Sometimes birds desperately without previous clinical signs (Allison *et al.*, 2005).

### **Neurotopic velogenic Newcastle disease**

Acute signs from the respiratory tract and nervous system dominate. Sudden depression, inappetence and drop in egg production are seen together with coughing and other signs from the respiratory tract, followed by nervous signs within a few days. Mortality is usually around 10-20% for adult birds but can be higher for young birds (Kommers *et al.*, 2002)

### **Mesogenic Newcastle disease**

Variable to high morbidity is evident in an exposed flock which will show moderate mortality characterized by nervous and respiratory signs. An acute drop in egg production occurs in susceptible mature flocks with the presence of shell-less eggs. Coughing and other signs from the respiratory tracts dominate. Other symptoms are depression, loss of weight and decreased egg production for up to three weeks. Signs from the nervous system can develop late in the disease (Kommers *et al.*, 2001).

### **Lentogenic New castle disease**

Acute onset with moderate to high morbidity. Mild to inapparent respiratory signs are noted but negligible mortality occurs in uncomplicated cases. Lentogenic ND may be responsible for asymptomatic drops in egg production in incompletely immunized commercial layer or breeder flocks. No nervous signs and mortality is usually negligible and often subclinical but mild respiratory signs and a small drop in egg production can be seen (Piacenti *et al.*, 2006).

### **Pathology**

Gross lesions: As with clinical signs, the gross lesions and the organs affected in birds infected with NDV are dependent on the strain and pathotype of the infecting virus, in addition to the host and all the other factors that may affect the severity of the disease. No pathognomonic lesions are associated with any form of the disease (Piacenti *et al.*, 2006).

### **Velogenic viscerotropic Newcastle disease.**

#### **Gross lesion**

The presence of multifocal hemorrhages seen through the serosal surface of the intestines, multifocal areas of necrosis and ulceration of the gut-associated lymphoid tissue, and disseminated foci of necrosis in the spleen are highly suggestive of VVNDV infection (Susta *et al.*, 2010). The cecal tonsils, which are especially prominent gut lymphoid aggregates located in the proximal portion of the ceca, are often regarded as the “old faithful” lesion for VVND, as they most consistently display hemorrhage and necrosis grossly. Perithymic hemorrhages are occasionally observed, and as the disease progresses, there is severe atrophy of thymus and bursa. Comb and wattle edema are variably present. Eyelid edema and hemorrhage are consistent findings in animals inoculated via the conjunctival route. They are markedly hemorrhagic and appear to result from necrosis of the intestinal wall or lymphoid tissues such as cecal tonsils and Peyer’s patches (Miller *et al.*, 2010.)

#### **Microscopic lesion**

The most unifying histologic feature is severe necrosis of the lymphoid tissues scattered throughout the body, most especially prominent in spleen and gut-associated lymphoid tissue, which corresponds to the foci hemorrhage and ulceration noted grossly (Alexander, 2003). In the less severe, or initial stages, there is lymphoid depletion and hyperplasia of macrophages with large vacuolated cytoplasm (commonly referred to as the “starry-sky” effect). In later stages, there is accumulation of cellular and karyorrhectic debris, pyknosis, and numerous macrophages with vacuolated cytoplasm that contain nuclear debris (Miller *et al.*, 2010.).

In the thymus, very early in the infection, there is almost complete necrosis of the cortex. The medulla usually has less severe lymphoid depletion. In the bursa of Fabricius, there is severe loss of lymphocytes both in the cortex and medulla of numerous follicles. The intrafollicular epithelial cells become prominent, and there is accumulation of numerous macrophages. Occasionally, there is formation of epithelium-lined cysts within the lymphoid-depleted lobules. Although numerous follicles are affected at the same times, it is not unusual to observe severely affected lobules adjacent to less affected or normal ones. Microscopic changes in the brain are minimal with VVND, even in

birds dying with neurologic signs. Perivascular cuffing is occasionally described (Alexander DJ, 2003).

### **Velogenic neurotropic Newcastle disease**

#### **Gross lesion**

Gross lesions are often absent, and the involvement of the visceral organs appears to be minimal, although animals euthanized in the early stages of disease may have splenic or proventricular congestion. Despite the neurotropism of these strains, gross lesions in the central nervous tissue are not present. In comparison to VVND, there are no characteristic gross lesions for VNND (Miller *et al.*, 2010).

#### **Microscopic lesion**

Histopathologic changes in chickens infected with VNND strains are largely restricted to the central nervous system. There is multifocal mononuclear perivascular cuffing, associated with hypertrophy/hyperplasia of vascular endothelium, moderate gliosis, and multifocal necrosis of the Purkinje cells. Other reported histologic lesions with VNNDV are lymphoid depletion, and myocarditis. No reports of documented pneumonia with VNNDV were found in the literature (Brown *et al.*, 1999).

### **Mesogenic Newcastle disease**

#### **Gross lesion**

Gross lesions with mesogenic strains are minimal. Chickens infected with mesogenic strains had mild splenomegaly and some degree of conjunctivitis when inoculated via eye-drop instillation. In the field, infection with mesogenic strains is often associated with secondary bacterial infections, which have their own set of morphologic correlates (Alexander DJ, 2003).

#### **Microscopic lesion**

Histological, there is a range of changes seen with mesogenic strains. The more virulent strains, those that cause a notable degree of clinical disease, consist mainly of nonsuppurative encephalitis that has many similarities to the cases caused by the VNND strains (i.e., perivascular cuffing and gliosis) (Susta *et al.*, 2010).

### **Lentogenic Newcastle Disease**

#### **Gross lesion**

Lentogenic strains produce mild pulmonary hemorrhages and splenomegaly was described. Lentogenic strains of NDV had been isolated together with *E. coli*, and gross lesions consisted mainly of tracheal hemorrhages when the same NDV isolate was experimentally inoculated into SPF chickens, no gross lesions were detected (Hooper *et al.*, 1999).

#### **Microscopic lesion**

Microscopic lesion of these strain are hyperplasia of the lymphoid follicles in spleen and air sacs were present, lymphoid follicle proliferation mainly in the lamina propria of the trachea. Some lentogenic isolates in Australia caused nonsuppurative tracheitis in association with *E. coli* field outbreaks, or, when experimentally inoculated in SPF chickens, induced mild changes, including lymphocytic infiltration, loss of cilia, and squamous metaplasia in the proximal trachea. Aerosol delivery of congestion, goblet cells hyperplasia, edema, and multifocal submucosal infiltration of scattered heterophils, lymphocytes, and plasma cells) (Hooper *et al.*, 1999).

#### **Diagnosis**

Clinical diagnosis based on history, signs and lesions may establish a strong index of suspicion but the laboratory confirmation must be done. Hemagglutination and hemagglutination inhibition test, virus neutralization test, Enzyme linked immunosorbent assay, plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of the ND virus. Now RT-PCR is the most exclusively used method to detect AIVs and NDVs. RT-PCR assay is more sensitive, specific and less labor intensives as compare to other conventional methods used for lab diagnoses such as virus isolation, Immuno-Fluorescence Staining, Neuraminidase Inhibition and ELISA. Using modern technologies, new diagnostic techniques are being developed for identification and differentiation of NDV strains. Other molecular diagnostic tests like real time PCR and nucleotide sequence analysis are also important in viral disease diagnosis (Kim *et al.*, 2007).

## Isolation and identification

### Direct detection of viral antigens

Immuno histologic techniques offer a rapid method for the specific demonstration of the presence of virus or viral antigens in organs or tissues. Immunofluorescence techniques for thin sections of trachea or impression smears and an immunoperoxidase technique for thin sections (Kommers *et al.*, 2003) have been reported and used in NDV infections.

### Virus isolation of NDV

Although molecular techniques, especially those developed to employ RT-PCR directly on samples from affected birds (Kommers *et al.*, 2003), mean that a positive diagnosis at least can be obtained rapidly without virus isolation, it is still important that, for primary outbreaks especially, the virus is isolated for proper characterization and future work.

### Culture system

Virulent ND viruses can be propagated in many cell culture systems, and viruses of low virulence can be induced to replicate in some of them. It is possible to use primary cell cultures or even cell lines for routine isolation of NDV. The embryonated chicken egg, however, represents an extremely sensitive and convenient vehicle for the propagation of NDV and is used almost universally in diagnosis. Embryonated chicken eggs should be obtained from a specific pathogen free (SPF) flock and incubated for 9-10 days at 37°C before use. If SPF eggs cannot be obtained, eggs from a flock free of NDV antibodies should be used. NDV strains in eggs containing yolk antibodies can be propagated, but the virus titer is usually greatly reduced, and such eggs should be avoided for diagnostic use (Kommers *et al.*, 2003).

### Serologic tests

Antibodies to NDV may be detected in poultry sera by a variety of tests including single radial immune diffusion, single radial hemolysis, agar gel precipitin and plaque neutralization. Sera from other species (including turkeys) may cause low-titer, nonspecific agglutination of chicken RBCs, complicating the test. Such agglutination may be removed by adsorption with chicken RBCs before testing. Although the HA

and HI tests are not greatly affected by minor changes in the methodology (Kuiken *et al.*, 1998).

### Real-time PCR

The advent of real-time PCR using fluorogenic hydrolysis probes provided highly sensitive and rapid testing procedures. Generally speaking, these types of procedures showed sensitivity limits in the same order of magnitude of the nested RT-PCR assays; increased specificity of the test was due to the application of labeled probes and reduced risks of contamination by avoiding post-amplification manipulations. The increased amount of genetic sequence data available particularly in the last decade has highlighted the high degree of genetic variability of APMV-1 and particularly of the F gene. This variability may well explain the occurrence of false-negative results provided by different probe-based real time RT-PCR protocols developed for this target the development of alternative protocols (Rue *et al.*, 2010).

### Public Health Important

Humans are among the many species that can be infected by NDV in addition to avian species. NDV may cause conjunctivitis in humans, when a person has been exposed to large quantities of the virus. Mostly, Laboratory workers and vaccinators are affected. The use of personnel protective equipment and biological safety cabinet has reduced the exposure of laboratory workers. Infection is rarely seen in the workers of a farm; moreover, persons handling or consuming poultry products do not appear to be at risk (Moscoso *et al.*, 2005). The conjunctivitis usually resolves rapidly, but the virus will be shed in the ocular discharges from 4 to 7 days. In some cases, mild, self-limiting influenza like disease with fever and headache has also been reported in humans. There is no evidence found to support human to human transmission but the potential for human to bird transmission exists (Miller *et al.*, 2009).

### Prevention

Vaccination. Conventional programs: Lentogenic infection of broilers can be prevented by day old administration of aerosol or eye drop vaccine using Hitchner B1 with subsequent boosters in drinking water or by the aerosol route. Administration of a preparation comprising live virus with complementary antibody (Newplex®) by the in ovo route at 18-days of incubation is protective in countries where the vaccine

is available. Recombinant pox and HVT-vector vaccines expressing the fusion (F) protein of NDV are available for either in ovo or subcutaneous vaccination. Lentogenic infection of breeders can be prevented by 10 day administration of Hitchner B1, by the aerosol or eye drop route. Subsequent vaccinations include 24 day, and 8 week Hitchner B1 or LaSota in non-chlorinated drinking water, followed by multivalent oil inactivated emulsion at 18-20 weeks (Wakamatsu *et al.*, 2006). An optional 45 week multivalent oil inactivated emulsion may be administered to boost maternal antibody transfer, depending on antibody titer of the flock, risk of exposure, and other factors relating to the operation. In areas with a defective cold-chain the V-strain live the most stable mutant ND can be distributed to subsistence and backyard flocks. A variety of vaccination programs can be followed depending on the risk of infection, virulence of agent, management system, and economic factors. In countries with endemic vvND, rigorous programs are implemented, incorporating day-old subcutaneous emulsion vaccine together with attenuated live vaccine by the eye-drop route. Hitchner or LaSota vaccine is administered to broilers by the aerosol route at 10 day intervals thereafter. Breeders may be immunized with mesogenic-strain vaccines in some countries (Stauber *et al.*, 1995).

## Conclusion and Recommendations

Newcastle disease is highly contagious and one of the most important animal diseases in the world, both for the number of animals affected every year and for the severe economic impact on the poultry industry. Current, efficient, accurate and reliable detection and confirmation of ND is important to limit economic losses and contain the disease. Newcastle disease is wide variety of disease presentations, it is important to enhance the awareness especially poultry farm industry as well as utilizing the most efficient and accurate laboratory testing procedures. Current laboratory testing is essential to confirm field suspicion, to identify the virus, and to comply with international reporting requirements. Based on the above conclusion the following recommendation will be forwarded.

- It will be seen that the foregoing review has been confined largely to its economic loss and its improvement.
- The relative merits of using virulent virus and current, efficient, accurate and reliable detection and confirmation of the virus will be minimize the huge economic loss due to the disease.
- Since Newcastle disease virus is a highly contagious disease, properly and efficiently follows the prevention and control mechanisms.

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