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Worldwide Sustenance and evolution of Newcastle disease virus in poultry (a review)

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ABSTRACT

Newcastle disease virus (NDV), a single-stranded RNA virus is the causal organism of a disease called Newcastle disease (ND), which is a major and continuous threat to the poultry industry in many developing countries around the world. The prevalence of the virus is very high all over the world and is contributing to serious economic losses to the poultry industry. Despite different vaccination strategies, Newcastle disease is still endemic in many countries across the globe. It produces nervous and respiratory signs which lead to a drop in egg production and mortality in birds. Considering the fact that vaccination is no longer yielding desired results it highlights the need to identify factors responsible for the never decreasing presence of the Newcastle disease virus worldwide. There are many factors such as diversity in classification, broad host range, worldwide distribution, genomic modifications and failure of detection which along with other factors are discussed in the present review. This article compiles those factors responsible for the continuous presence of disease worldwide with the aim to understand and eliminate those factors to decrease its prevalence.

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INTRODUCTION

Newcastle disease is an infectious and contagious disease and its causal organism NDV belongs to the genus *rubulavirus* of family *paramyxoviridae*. These viruses are distinguished on the basis of envelope and a negative-sense, single-stranded RNA genome (Barnezange and Jestin, 2005). Newcastle disease virus has been grouped into three strains *i.e.* velogenic, mesogenic and lentogenic in decreasing order of its virulence, this grouping is done based on its pathogenic and virulence properties (Haque *et al*., 2010). Newcastle disease is significant in the poultry industry. It is worldwide in distribution and has been responsible for destructive outbreaks globally which led to considerable morbidity and mortality. Newcastle disease is a highly contagious disease which not only affects poultry but other bird's species as well, irrespective of sex and age of the bird (Haque *et al*., 2010). A morbidity and mortality rate of 90-100% is the cause of major economic losses (Gohm *et al*., 2000). Among all bacterial, viral and parasitic diseases, ND is regarded as the serious threat to commercial poultry industry (Rehman *et al*., 2013) involving the liver injury as well. According to the Office International des Epizooties (OIE), Virulent NDV is categorized in list A disease and is dealt according to international regulations (Aldous *et al*., 2003). Newcastle disease virus has a genomic length of approximately 16 kb. The genome encodes for six proteins namely phosphoprotein (P), hemagglutinin-neuraminidase (HN), nucleocapsid protein (NP), fusion protein (F), a large RNA-dependent polymerase protein (L) and matrix protein (M) (Haque *et al*., 2010).

There are many factors which contribute to the virulence of ND virus but the major factor responsible for changes in virulence is the F protein cleavage site. A specific cleavage site motif of F protein is found in mesogenic and velogenic strains and proteases cause its cleavage which results in systemic infection. Lentogenic strains on the other hand consist of a different motif and trypsin-like proteases are responsible for its cleavage (Farooq *et al*., 2014; Masoud *et al*., 2022). During replication, virus genome can evolve very rapidly because of the errors introduced by its machinery allowing it to customize to the changing environment which may lead to severity in signs and symptoms of disease and difficulty in disease diagnosis (Munir *et al*., 2012). There are many factors which contribute to the sustenance and evolution of NDV throughout the world, these factors are discussed below.

Factors for sustenance and evolution of Newcastle disease virus

Broad host range

Not only wide range of birds but animals are also infected by ND virus, with those infected include wide variety from reptiles to humans (Awan *et al*., 1994). Newcastle disease virus is responsible for establishing infections in some 241 bird species, these bird species accounts for 27 orders out of the total 50 included in this class (Awan *et al*., 1994). There is a possibility that all birds are prone to infection from ND virus, but disease can vary from species to species (Awan *et al*., 1994).

Wild Birds

Newcastle disease virus has been isolated from migratory birds such as waterfowl and other aquatic birds, but these isolates are mostly less virulent in chicken (Fig. 1). Virulent isolates from a ND outbreak in Ireland were uncommon, but they were associated with avirulent viruses which are commonly linked with waterfowl (Alexander *et al.,* 1992). On the basis of these findings it can be concluded that, mutation can change avirulent viruses to virulent viruses. Whenever virulent ND viruses have been isolated from wild birds, domestic birds were also suffering from ND infection in same area (Alexander, 1995). In North America there had been an epizootic because of virulent ND virus affecting cormorants (Wobeser *et al.,* 1993).

Caged Birdsi

Virulent strains of ND virus had been isolated from birds which are held captive (Senne *et al.,* 1983). It is highly unlikely that infection in caged birds is because of the infected feral birds in the countries from which they originated; infact holding stations where birds are kept before export are most probable cause of infection (Awan *et al*., 1994). In 1991 severe ND outbreaks involving pet birds were reported in six states in USA (Panigrahy *et al*., 1993). It was assumed that the virus was introduced as result of illegal imports.

Domestic Poultry

Virulent strains of ND virus were isolated from all kinds of commercial poultry, which include wide variety of birds stretching from pigeons to ostriches (Fig. 1).

Racing and Show Pigeons

A strain of ND virus appeared in pigeons in 1970, which differed antigenically from classical strains and most probably it was of Middle East origin. In racing pigeons ND was first reported in 1981 in Italy (Biancifiori and Fioroni, 1983) which then leads to a panzootic and ND spread worldwide among show and racing pigeons.

Diverse classification

One of the major reasons for persistence of APMV1 is the diversity of this virus. According to latest classification system APMV1 isolates are divided into two classes namely class I and class II.

Class I

The isolates of this class share significant similarity among its genome thus are placed under single genotype called genotype I (Diel *et al*., 2012). Based on amino acid motif present at the cleavage site of fusion protein, all the isolates of class I are considered to have low virulence except the one (Glickman *et al*., 1988; Masoud *et al*., 2022).

Class II

Those isolates which come under this class are divided into 18 genotypes (Diel *et al*., 2012; Courtney *et al*., 2013; Snoeck *et al*., 2013; Susta *et al*., 2014).

There are four genotypes of class I namely I, II, III, IV which are considered historic because these emerged between 1930 and 1960. These genotypes comprise of 15,186 nucleotides. Contrary to this, genotypes which emerged after 1960 have 15,192 nucleotides. These include genotypes V, VI, VII, VIII, IX and X-XVIII (Czeglédi *et al*., 2006; Miller *et al*., 2010).

Transmission

Transmission of ND virus is rapid in commercial poultry houses, but it is less rapid within a village flock and between village flocks. It usually takes weeks for ND virus to spread in a flock and to spread in a village month are required (Awan *et al*., 1994). ND virus is mainly transmitted by respiratory route in intensive poultry farms. After establishment of infection both fine and large droplets are formed as a result of virus shedding in atmosphere, these droplets transmit infection to other birds. This mode of transmission is significant in intensive poultry houses (Awan *et al*., 1994). In village poultry predominant mode of transmission of ND virus is oral route, as feces are eaten up by poultry (Awan *et al*., 1994). It is reported that in Great Britain the major cause of 1980s outbreaks was feed contaminated with infected carcass of feral pigeons and feces (Alexander, 1995). The penetration of eggshell by ND virus might happen after laying that's why the data on vertical transmission is ambiguous. Although, the death of infected embryo occurs significantly before hatching. Avirulent strains can also infect eggs; such eggs can hatch properly (Alexander, 1995).

Geographic distribution

A major factor contributing to the existence of NDV is its worldwide distribution. Currently, genotypes V, VI, VII and VIII which belong to the class II are the major source of outbreaks worldwide. Considering all these genotypes, viruses of genotype VI emerged in 1960 and remained the most dominant viruses in Asia till 1985 (Mase *et al*., 2002). Later on, genotype VII became the most predominant in that region. This genotype has eight subgenotypes (VIIa–VIIh). Among those subgenotypes, five genotypes (VIIa–VIIe) are representative of isolates from Kyrgyzstan, China, Kazakhstan and Malaysia (Bogoyavlenskiy *et al*., 2009; Wang *et al*., 2006) and remaining three (VIIf–VIIh) have isolates which are found in Africa (Snoeck *et al*., 2009).

The genotype VII particularly VII e of class II has the greater prevalence in Asian countries particularly in Pakistan and Indonesia (Xiao *et al*., 2012). Some recent major outbreaks in wild birds and poultry are because of viruses which belong to V, VI and VII genotypes. These genotypes contain only virulent viruses which are isolated

from different continents because they are extremely mobile. Contrary to this some virulent viruses of other genotypes such as XI, XIII and XVI are found in Madagascar, Southwest Asia and North America respectively. While genotype XIV, XVII and XVIII are predominantly found in Africa. These viruses have narrow distribution geographically and these viruses were mostly isolated from poultry.

Evolution/Genetic modification

Generally, class I viruses are considered as viruses of wild birds but the existence of viruses genetically identical to class I viruses and isolation of these viruses from live bird markets (LBMS) supports the fact that the viruses in these populations have epidemiological connections. (Kim *et al*., 2007b) suggested that class I viruses circulate in both wild birds and LBMS worldwide. According to results of a phylogenetic analysis, viruses of low virulence can become virulent gradually (Gould *et al*., 2001). Increased virulence of a ND virus is because of transformations in cleavage site of fusion gene. There is a close genetic relation between virulent viruses and those of low virulence circulating in Australia (Kattenbelt *et al*., 2006). With only two-point mutations, a virus of low virulence can be changed to a virulent one (Westbury, 2001).

Zoonosis

Newcastle disease virus can also infect humans; predominant signs include conjunctivitis with shedding of virus (Awan *et al*., 1994). In UK according to the Advisory Committee on Dangerous Pathogens NDV is placed in hazard group 2 (Fig. 1). In humans the first case of NDV infection was reported in a laboratory worker with signs of conjunctivitis. The reason was the accidental introduction of allantoic fluid infected with NDV into the eye (Burnet, 1943). Most of the cases of humans being infected with ND virus result from direct inoculation into the eye, vaccine handlers and laboratory workers were mostly affected by ND virus (Lippmann, 1952). Newcastle disease virus infections are not life threatening and usually not last for more than two days. Commonly reported clinical signs in human infections include conjunctivitis, excessive lacrimation, sub-conjuctival hemorrhage, edema of eyelids and reddening of eye (Capua and Alexander, 2004). In some rare cases symptoms include, fever, headache, chills with presence or absence of conjunctivitis (Capua and Alexander, 2004). Newcastle disease virus is transmitted to humans through direct contact with infected birds, with virus or with the carcasses of diseased birds. This transmission to humans leads to continuous circulation of the virus.

Contaminated poultry products

Both poultry products and infected carcasses can play a vital role in transmitting ND in poultry (Alexander, 1995). In case of village poultry, diseased birds are often used as a food by farmers while the viscera of the infected birds are usually fed to dogs, cats and poultry (Awan *et al*., 1994). There is a common practice of throwing offals into the field which results in spreading infection (Fig. 1).

Species other than poultry

Newcastle disease virus can infect some other species of poultry which can play role in transmission of NDV in poultry industry. Species which are infected by NDV include turkey, guinea fowl, ducks, pheasants, geese, peacocks and doves (Awan *et al*., 1994). Despite being more resistant to infection by NDV, disease has been reported in ducks and geese. When oral and cloacal swab samples were taken from apparently disease-free ducks in Hong Kong, 3% of the samples yielded NDV with majority of it isolated from cloaca (Awan *et al*., 1994). In villages rearing of chickens is done in close contact with geese and ducks which is a potential cause of virus spread between species (Fig. 1).

Involvement of non-avian species

Some species of animals like foxes, dogs, rodents and cats uptake NDV while eating infected carcass. These animals then can shed virus in feces even up to 72 hours after eating carcass (Awan *et al*., 1994). As these animals meet village environment and poultry, these animals can play a vital role in transmission of NDV.

RESERVOIRS

Chicken as Reservoirs

Birds having latent infection and those which survive natural infection yet still carrying the agent usually are the reservoir of ND and Avian Influenza infection. Birds which recover from infection usually become carriers but usually for a small time period. The fact that chickens becoming carriers for longer period is unclear. Vaccinated chicken can excrete virus for almost 5 weeks and there is a possibility of virulent virus causing mild infection in vaccinated flocks (Awan *et al*., 1994). Addition of new birds in a flock through hatching coupled with some birds evading infection during an outbreak will always lead to presence of susceptible birds in village which can get infection from disease birds. The fact that, there will always be some birds without infection leads to the maintenance of infection in a cyclic manner and for a long period of time.

Inanimate Reservoirs

Newcastle disease virus is shed in feces of bird and it can survive in tropical conditions for almost 8 weeks, with temperature as high as 40°C (Awan *et al*., 1994). At mild temperature ranging from 20 to 30°C it can survive for almost 3 months and at cooler temperatures its survival rate increases significantly (Awan *et al*., 1994). The feces with other materials act as a reservoir for ND virus.

Wild Birds and Migratory Birds

Wild birds are thought to play a vital role in transmission and evolution of ND virus (Jindal *et al*., 2009; Kim *et al*., 2007a). The studies of (Miller, 2015) discovered that there is a spread of virulent ND virus across continents between three countries having no geographical connection. There was a rapid spread of sub-genotype VIIi of ND virus in Asia and Middle East which lead to outbreaks in pets, wild birds and vaccinated birds (Rehmani *et al*., 2015). In Ukraine wild bird surveillance for ND concluded that during autumn migration, virus isolation rate was high,

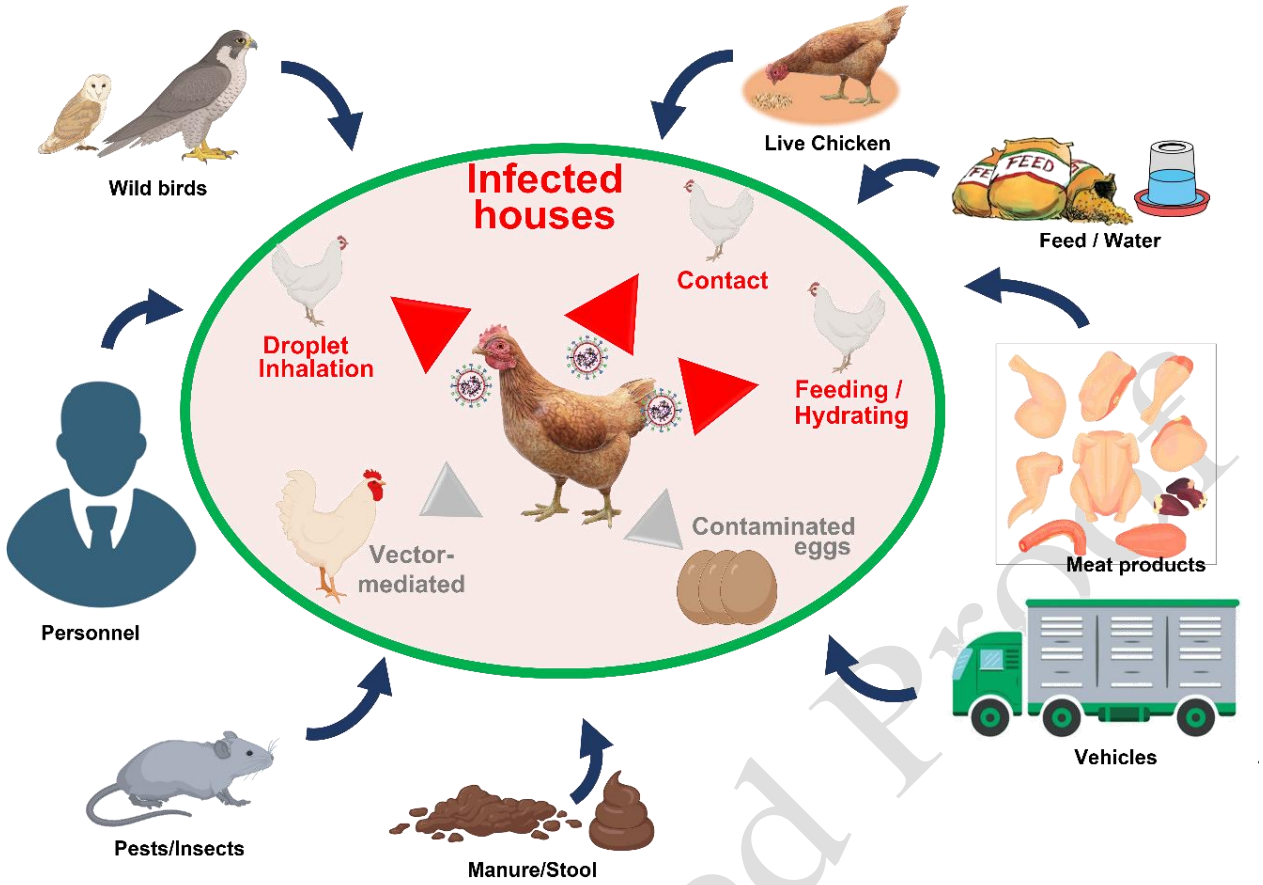

Fig. 1: The patterns and vehicles involved in Newcastle disease virus transmission and sustenance in the environment abstracting that the virus is circulated among wild birds, live chickens, feed/water, vehicles, manure/stools, pests and personnel involved with the poultry farms

and it was isolated from teals, widgeons, mallards and dunlins (Muzyka *et al*., 2014). During winter rate of isolation was less with virus mainly isolated from white-fronted geese, shelducks, starlings and mallards (Muzyka *et al*., 2014). This data supported the fact that there is a possibility of intercontinental transmission of ND virus by wild birds through Africa-Europe route. To study north-south and east west intercontinental transmission of virus swab samples were taken from migratory birds and analyzed phylogenetically, most of the isolates were closely related to the ND virus isolates previously reported in wild birds (Kim *et al*., 2007a). Study conducted in Russia to evaluate ND strains in domestic and feral pigeons demonstrated some Serbian strains in the country (Pchelkina *et al*., 2013). This study leads to the conclusion that despite geographical borders pigeon viruses do travel across large distances.

Replacement of old strain with new

In Kazakhstan ND was reported in 1980 and despite vaccination programs at regular intervals it is considered as endemic in domestic poultry. The strains of virulent NDV isolated from poultry from Kyrgyzstan and Kazakhstan between 1998 and 2005 had fusion protein cleavage site which was identical to that of a virulent strain. The ICPI value ranged from 1.05 to 1.87 (Bogoyavlenskiy *et al*., 2009). It has been reported that genotype XIII ND virus which existed previously in Pakistan during 2009-2011 has now been replaced by sub-genotype VIIi and it has become the most predominant sub-genotype since 2012 (Miller, 2015). Similarly, sub-genotypes VIId and VIIb, which existed previously in Israel have been replaced by sub-genotype VIIi.

Failure of detection

Frequently used assay in United States for detection of NDV include real-time reverse transcription polymerase chain reaction (RRT-PCR) sometimes it fails to detect ND virus from waterfowl, which is of low virulence. Similarly assays based on fusion gene for detection of some virulent genotypes of NDV sometimes fail in detection of virulent NDV (Miller *et al*., 2010). Another method for detection of NDV is to replicate these viruses in embryonating chicken eggs (ECE) and time required to kill the embryo which is known as mean death time (MDT) varies according to the virulence of virus. Furthermore for NDV identification Hemagglutination Inhibition Test (HI) test can be used. Characterization of ND virus can be done using monoclonal antibody (mAb), for this purpose a group of of mAb is used to differentiate varying strains of virus**.** But using mAb assay as a tool for rapid characterization of class I viruses is not optimum because these mAbs were made for the recognition of class II viruses (Collins *et al*., 1998; Kim *et al*., 2007b). Different probes and primers are designed for detection of NDV based on matrix (M) gene a highly conserved region in viral genome. It detects most of the genotypes of class II viruses but fails to detect class I viruses because of their heterogeneous genetic nature (Kim *et al*., 2007a).

Biosecurity
Lack of biosecurity measures are also contributing to the persistence of ND. Newcastle disease virus can be transmitted by man visiting different farms and different countries. Virus can be transmitted by an infected man or the one mechanically carrying the virus, the former, however is not very common. Alexander (1995) discussed the role of man in transmission of ND virus in poultry industry. In village poultry, vaccinator, health workers, farmers and their families can transfer ND virus between villages and farms as they come in contact with the virus.

Use of bird faeces as fertilizers
Newcastle disease virus is shed in feces; these feces are used in many areas as a fertilizer. The use of feces from diseased chicken is the source of virus spread in poultry (Alexander, 1995) and it is a contributing source of ND in village poultry.

Simultaneous infections with other pathogens
In case of village poultry, birds are exposed to a combination of infections which are caused by parasites, bacteria and viruses. In poultry problem of ecto and endoparasites is persistent which makes poultry viable to many other infections, leaving debilitating effects on poultry (Awan *et al*., 1994). Certain fungi produce aflatoxins which are ingested by poultry with feed thus causing stunted growth, high mortality and less feed intake, thus increasing the chances of ND. Another factor contributing to ND is coccidiosis which is among the major parasitic diseases of poultry and cause high mortality. The control of parasites as a part of ND vaccination program was a crucial step in controlling ND in Burkina Faso (Awan *et al*., 1994). In village poultry immunosuppression could be the result of vitamin A deficiency or infectious bursal disease (Awan *et al*., 1994), thus making poultry more prone to ND.

Breed susceptibility
According to a serological survey based on determining prevalence rates of antibody for ND virus, there is no likelihood of breed-specific infections in the case of ND virus in backyard, free range and intensive systems (Ezeokoli *et al*., 1984). According to (Awan *et al*., 1994) in Hong Kong ND susceptibility does not change in imported and local breeds. Contrary to this (Awan *et al*., 1994) said that imported breeds of broiler and layer are more prone to ND as compared to the local breeds of chicken in Thailand. In Taiwan the resistance against ND infection is higher in indigenous fowl as compared to white leghorn breed (Awan *et al*., 1994). Indigenous poultry breeds across the globe may vary in their susceptibility against ND infection (Awan *et al*., 1994)

Vaccination Failure/Lack of Cross Protection
There is a large difference between vaccines and current circulating virulent strains both antigenically and phylogentically which can result in evolution of virulent ND virus (Miller *et al*., 2007; Masoud *et al*., 2022). Prevailing vaccines can provide immunity against disease but are unable to stop shedding of virus (Kapczynski and King, 2005; Miller, 2009; Utterback and Schwartz, 1973). Recently, evidence that the use of vaccines having matched genotype can reduce shedding of virus is emerging. In recent times use of vaccine having same genotype as that of challenging strain demonstrated increased ability of vaccine to control shedding of viruses of genotype VII and V, respectively (Hu *et al*., 2009; Miller, 2009). Some variants isolated from field that escape vaccination have been reported (Cho *et al*., 2008). A comparison of killed vaccine based on matched HN gene and commercial vaccine showed equal protection against morbidity and mortality, with former proving to be more effective in controlling decrease in egg production (Cho *et al*., 2008). Moreover, according to a study *Moringa oleifera* seed meal might enhance immunity against ND.

Conclusion
Newcastle disease is a highly contagious and infectious disease and is endemic throughout the world. It is one of the major limiting factors in poultry production and has been causing significant mortality in poultry industry. It has a broad host range including not only poultry but some wild birds, migratory birds and animals as well. Currently, major problem regarding this disease is its sustenance across the globe despite extensive research and documented knowledge in the concerned field. Involvement of migratory birds, genetic modification and lack of cross protection are among the many factors which are contributing to its continuous existence and circulation all over the world. Other factors responsible for its persistence include wide range of hosts, worldwide distribution, diagnostic challenges, concurrent infections and lack of biosecurity measures especially in developing countries. To decrease prevalence of Newcastle disease these factors are needed to be scrutinized besides village poultry should be given appropriate attention. Efficient monitoring and surveillance strategies along with some rapid diagnostic methods are required for the control and eradication of Newcastle disease.

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UI and FM undertaken the final write up, IA and AZ finally proofread the article

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