Review Article

Potential Economic Impact of Newcastle Disease Virus Isolated from Wild Birds on Commercial Poultry Industry of Pakistan: A Review

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Abstract | Newcastle disease (ND), caused by avian paramyxovirus-1 (APMV-1) is an important disease of avian species and continuously cause outbreaks in commercial poultry throughout the world. Despite intensive vaccination, ND is endemic in Pakistan and locally known as Ranikhet. Wild birds are considered natural reservoirs of Newcastle disease virus (NDV) and some common resident or migratory wild birds are associated with outbreaks of ND in commercial poultry in Pakistan. Continuous isolation of new genotypes in Pakistan shows the evolving nature of the virus and the emergence of new strains is limiting in its diagnosis and control. Pakistan is a developing country and poultry industry is the backbone of its economy. Recent outbreaks have caused huge losses to the poultry industry. This review details the possible role and potential of some common captive and non-captive wild bird species in the spread of NDV and also its economic impact on the commercial poultry industry of Pakistan.

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Introduction

Newcastle disease (ND) is an acute and highly fatal viral disease which affects a large number of domestic and wild bird species (Zhu et al., 2010). In the local language, ND is known as Ranikhet in Indo-Pak subcontinent (Ravindra et al., 2009). Since its first identification in 1926, ND still poses a great economic threat to the poultry industry as it causes high mortality and production losses (Alexander, 2001). Newcastle disease virus (NDV) or avian paramyxovirus-1, belongs to family *Paramyxoviridae* and genus *Avulavirus*. Recently, the nomenclature of all avian paramyxoviruses including NDV of family *Paramyxoviridae* have been amended by International Committee on Taxonomy of Viruses and they are renamed as avian avulavirus (AAvV) (Wajid et al., 2017). Nine serotypes of *Paramyxoviruses* (APMV-1 to APMV-9) have been isolated from avian species. NDV is single-stranded, negative-sense enveloped RNA virus (Mayo, 2002) with almost 15 kb genome size and codes for six structural proteins (Choi et al., 2010). F protein is most important as it involves in the binding of the virus to host cell required for initiation of infection (Pham et al., 2005). OIE acknowledges reporting the F cleavage sequence as a primary factor for the determination of NDV virulence (Boynukara et al., 2013).

Commercial poultry is highly susceptible to NDV and several outbreaks have been reported in commercial poultry flocks worldwide and cause annual losses in millions of dollars (Shabbir et al., 2012). The continuous evolution of NDV and replication in poorly vaccinated birds suggest high environmental load with still unknown mechanisms. It is present in almost six continents of the world including Asia (Miller et al., 2010). In the last major outbreak of ND in the USA, death of about 4 million birds caused loss of approximately 162 million US dollars (Cattoli et al., 2011). Pakistan poultry industry is the second largest industry after textile with US$ 2 billion annual turnovers. The poultry industry is the backbone of the economy and employs approximately 1.5 million people in the country (Chaudhry et al., 2015). In Pakistan, recent NDV outbreaks in commercial poultry in 2012 caused huge losses to the industry. NDV has killed approximately 45 million poultry birds alone in Punjab resulting loss of 6 Billion (PKR). In 2012, NDV outbreak occurred in Jallo Wildlife Park in Lahore and caused the death of 190 peacocks (Hussain et al., 2015).

Identification of various factors involved in endemicity NDV is important for controlling the disease. Various reports suggest the involvement of wild in the spread of NDV and wild waterfowl is considered as a natural reservoir of APMV-1 (Zanetti et al., 2005). Virulent NDV has been reported in various captive and non-captive avian species of Pakistan, but their potential in the spread of NDV is still unknown. Involvement of resident and migratory wild birds in the spread of NDV in the country has always considered as a mystery (Shengqing et al., 2002). Every year during the winter season, wild birds migrated to Pakistan from different countries like Europe, Russia, Central Asian States and India (Shirazi, 2010) and considered a major source to spread NDV in Pakistan.

Newcastle disease virus

Newcastle disease virus (NDV) is a member of the superfamily *Mononegavirales,* genus *Rubulavirus* and family *Paramyxoviridae,* which is further classified into two subfamilies, *Paramyxovirinae* and *Pneumovirinae.* Recently, the nomenclature of all avian paramyxoviruses including NDV of family *Paramyxoviridae* have been amended by International Committee on Taxonomy of Viruses and they are renamed as avian avulavirus (AAvV) (Wajid et al., 2017). NDV is negative sense, single stranded, non-segmented, enveloped RNA virus with 15,186 kb (15.2 kb) genome (Zhang et al., 2012). NDV encodes six structural proteins (NP, L, F, M, HN and P) in 5´ to 3´ direction (Alexander, 2001). Fusion (F), Matrix (M) and hemagglutinin-neuraminidase (HN) are important for virus binding and fusion with the host cell membrane and initiation of infection (Pham et al., 2005). The pathogenicity difference among various NDV strains is attributed to differences in the cleavage site within the F protein (Munir et al., 2012). OIE acknowledge F protein cleavage sequence as the primary determinant of virus virulence (Boynukara et al., 2013), which forms the basis of molecular assays for diagnosis (Rue et al., 2010). Lentogenic viruses contain ^{112}G-R/K-Q-G-R↓L^{117}, a monobasic motif at F protein cleavage site while velogenic and mesogenic viruses have multi-basic (^{112}R/G/K-R-Q/K-K/R-R↓F^{117}) amino acid sequence (Miller et al., 2010). As few as two nucleotide mutations can cause conversion of low-pathogenic strains to virulent form; however, only a few such cases are documented yet. Such mutations were involved in virus outbreaks that occurred in Australia (1998-2000) and Ireland (1990). Less virulent viruses in coastal wildlife populations of Ireland were endemic, and also initially detected circulated in Australian poultry industry (Alexander et al., 1992).

NDV strains are classified in two classes; class I (avirulent in chickens) and class II (virulent strains of NDV). Class II strains are further classified into 15 genotypes (I–XV). Genotypes VI and VII are genetically diverse and further divided into eight (A-H) and five (A-E) sub-genotypes, respectively. Cless I strains have been isolated mostly from waterfowl worldwide (Czeglédi et al., 2006; Munir et al., 2012).

Historical perspective and current situation of disease

All available data on Newcastle disease tells about the occurrence of the first outbreak of Newcastle disease (ND) dates back to 1926 reported at two geographically different locations of the world, Java an island of Indonesia and in Newcastle town of England (Doyle and Minett, 1927). However, some evidence showed that outbreaks similar to ND may have occurred before first identification in 1926. Most important statement regarding ND outbreak before 1926 was given by (Macpherson, 1956). He believed that an unidentified mysterious disease in Western Isles of Scotland in 1898 causing the death of all domestic fowl was ND. John Camp- bell reported this outbreak in a Gaelic poem, Call nan, Caero (The

loss of the hens). It was observed that ducks remained unaffected while disease killed all domestic fowl, this would apply equally to ND and HPAI (Alexander, 2001). After 1926, outbreaks of highly virulent ND had been reported worldwide including India, Japan, Kenya and Australia. In 1952, it was reported in Syria, Palestine, Europe, Sicily Island and the USA. In the 1960s, ND was reported in Canada, Central and South America, China, Mexico and whole Europe as 2nd and 3rd panzootics. These outbreaks were credited to trade of exotic psittacine birds, rapid industrialization of poultry and ubiquitous presence of pigeons without application of any quarantine measures (Alexander, 1988). Still, it poses a threat to commercial poultry industry with nearly four panzootics have been identified (Miller and Koch, 2013). ND not only a constant threat to the livelihood of people attached to the poultry industry but affect human welfare by reducing food supplies (Alders, 2014). Vaccination coupled with strict biosecurity measures is the available best method to control ND (Miller and Koch, 2013). These measures emphasis on All in-All out system of production, restricted movements of personals and controlled movements of birds to reduce virus contact with birds. Currently, vaccination in combination with rapid diagnostic approach and culling of the infected flock is used for the containment of ND around the world. From the 1950s to 1990s attenuated live and inactivated ND vaccines were used to decrease morbidity and mortality to reduce economic losses of disease (Gallili and Ben-Nathan, 1998). In recent years, the number of reported cases of ND has increased with high economic losses. In Pakistan, intensive vaccination is routine practice in poultry to avoid the occurrence of disease however ND is endemic in poultry including commercial broiler and layer (Farooq et al., 2014). NDV has killed 45 million broiler chickens resulting economic loss of 6 billion PKR during 2011-2012 only in Punjab province of Pakistan. NDV outbreak in 2012 in Jallo wildlife Park, Lahore-Pakistan killed 190 peacocks within a week (Hussain et al., 2015). Within three weeks, ND outbreak in seven districts of Sindh province of Pakistan cause death of about 167 wild peacocks (Munir et al., 2012).

On average, 60 countries around the world reporting outbreaks of ND from 2013 to 2015 and this increasing number shows expanding NDV genetic diversity. This suggests that vaccine strains (mainly genotype 1 and 11) are almost seven decades old and are genetically distant (18.3% to 26.6% nucleotide distance) from currently circulating strains of NDV (Dimitrov et al., 2016). This genetic distance between the vaccine and existing ND strains prevents the reduction of virulent virus shedding from the vaccinated flock (Miller et al., 2010). NDV is evolving like other RNA viruses. In 1945, inactivated vaccines became commercially available but not adopted much due to the high cost and not sufficiently able to prevent the spread of disease. In 1948, the first live vaccine made from virulent strains was commercially available and was only applicable to at least four-week-old chickens (Goldhaft, 1980). Within two years of this, two new strains of low virulence (LaSota and B_1) were isolated in the USA and approved for use in the market (Hitchner, 1975). Vaccination against ND is importantly considered only in the poultry sector. However, more than 236 species of birds are reported to be susceptible to NDV infection (Kaleta and Baldauf, 1988). Other than chickens, different resident and migratory wild birds including pheasants, pigeons, waterfowl, psittacine and cormorants are susceptible to high and less virulent strains of NDV.

Any outbreak from virulent ND whether mesogenic or velogenic is mandatory to report it to OIE and its consequences may result in blockade of poultry products imports from partner countries. The World Livestock Disease Atlas from 2006 to 2009 surveyed 176 countries included in OIE Animal Health Yearbooks and reported ND as the fourth most important problem of poultry industry after highly pathogenic avian influenza, infectious bronchitis and low pathogenic avian influenza. ND ranked 8th out of 71 diseases evaluated for the number of wild animals lost through disease or slaughter. During recent years, an increased number of outbreaks all around the world indicates that current vaccination practices are not enough to control the disease. Counties like South Africa, Pakistan, Iran, Vietnam, China, South Korea, Sweden, Romania and Israel were mostly affected during 2006-2009 (Dimitrov et al., 2016). From 2008 to 2010, ND outbreaks in domestic poultry were confirmed in 77 countries while 68 and 56 countries reporting ND outbreaks in 2013 and 2015 respectively.

Epidemiology and transmission of Newcastle disease in wild birds

Newcastle disease is a highly fatal disease of poultry with diverse clinical picture ranging from sub-clinical

infection to almost 100% mortality. ND is renowned for killing a huge population of rural poultry birds annually in Asia and Africa (Bell and Mouloudi, 1988). This variation in the clinical picture of the disease is mainly due to the type of strain involved, bird's species and their immune status (Shabbir et al., 2012). ND most commonly occurs from November to March (Abdu et al., 2005). ND is present worldwide and the major reason of disease spread is thought to be aquatic birds (Shengqing et al., 2002). First panzootic of ND was reported in the 1920s and within the next 30 years, it spread all over the globe. A second panzootic was reported in the late 1960s in the Middle East and global spread occurred within a decade due to highly intensified poultry farming. Psittacine birds carrying ND that were captured in wild were thought to be associated with second panzootic. In the late 1970s, third ND panzootic spread occurred which was related to doves and pigeons of *Columbidae* family (Alexander et al., 1997). NDV is present in almost 241 species of 27 to 50 orders of birds and wild birds are the natural reservoir of ND (Kaleta et al., 1985) and some most commonly infected species are pigeons and doves (*Columbia livia*), turkeys, ducks, house sparrow, partridges, pheasants, peafowl and dove. Usually, virus strains originated from wild birds are of low virulence but due to mutation they may be converted to the highly virulent virus in chickens (Snoeck et al., 2013). Waterbirds are important regarding the epidemiology of NDV due to their ability to spread virus to long distance through migration (Jørgensen et al., 2004). Data available on the geographical spread of NDV genotypes to other locations especially in South-East Asian countries is not sufficient for epidemiological investigations. Hence it is a basic requirement to characterize virus isolates in such regions if we want to establish an epidemiological link between spread to disease to new locations (Munir et al., 2012). Pakistan is a major exporter of wild birds like parrots to European pet bird's market. Recent studies have revealed the presence of exotic Newcastle disease strains in parrots and finches imported to Italy from Pakistan (Trust, 2011) which emphasis on strict monitoring of wild birds trade to different countries from areas of endemic NDV. Some evidence suggested that water birds are the natural reservoir of class I and class II genotypes 1 and X strains which are avirulent (Jindal et al., 2009). Geese are considered to be susceptible to ND infection and there are many reports of clinical signs of ND in China (Huang et al., 2004). Feed contamination by pigeons result in 20 outbreaks reported in1984 in unvaccinated flocks in the UK (Saif, 2003). Neurotropic ND has been reported in double-crested cormorants (*Phalacrocorax auritus*) many times (Allison et al., 2005). Wild waterfowl harbor nonpathogenic strains of NDV and virulent strains isolated from wild birds had caused outbreaks in commercial chickens a number of times. Although no direct epidemiological link was found but on the basis of strain similarity, early 1970's outbreaks in commercial chickens of south California (USA), were thought to be associated with psittacine originated strains of NDV (Walker et al., 1973). Similarly, cormorants developed widespread ND attributed to neurotropic virulent viruses during 1990–1992. Geese are considered to be susceptible to NDV infection and there are many reports of clinical signs of NDV in China (Huang et al., 2004).

NDV is transmitted through horizontal route only (Sharif et al., 2014). Ingestion or inhalation by direct contact with affected bird's secretions is the main route of disease spread. During incubation period (2-15 days) and course of clinical disease, affected bird's continuously shed virus in their feces and respiratory secretions which cause feed and water contamination and is one of the reasons of bird-bird disease spread. Some reports suggest virus shedding even during the convalescence period (Shabbir et al., 2012). True vertical transmission of virulent strains of NDV is not known, however chicks may get infected with contaminated eggs in hatcheries. Transmission through mechanical vectors like flies is also not known (OIE, 2009). However, some reports suggest the role of flies in the spread of NDV. Recently during an outbreak in backyard poultry in the U.S.A, an exotic NDV strain was identified and isolated from common houseflies (Chakrabarti et al., 2007). Newcastle disease virus particles have been found in the air up to a distance of 64 m downwind of disease infected premises which indicates air born spread of the virus to short distances (Hugh-Jones et al., 1973). ND spread through the air had extensively considered significant during 1970-1972 epidemic in the UK (Alexander, 1988). (Lancaster, 1966) established different modes of disease spread. They included the movement of live feral, exotic and game birds, movement of people, equipment and poultry products, contaminated feed and water. Involvement of these modes of transmission depends upon situations of rearing of poultry birds. Water sources like ponds and canal, especially in rural areas, are important regarding the spread of viruses as

they attract wild birds. Wild birds can contaminate the poultry sheds in areas where they have free access and act as vectors (virus shedding through feces) and are a source of spreading new viruses. Chances of virus spread to commercial poultry are increased if poultry rearing areas are in close proximity to wild bird's habitats like ponds (Si et al., 2013). However, in countries where birds are reared in environmentally controlled houses, there are fewer chances for wild bird's contact with poultry and transfer NDV as compare to those birds reared in villages or open areas which are more likely to be affected by virus strains carried by wild birds. Unusual movement pattern showed by migratory wild birds during early 1997 proposed their putative role in introduction of virulent NDV in UK domestic poultry (Saif, 2003). Provision of clean water to poultry is an important consideration as the virus can survive in water and its role in the spread of the disease has been reported but has given no significant importance (Awan et al., 1994).

Pigeons and doves (*Columbidae*): Pigeons are one of the important domesticated birds kept by humans for different purposes like food and hobby (racing). Out of different diseases affecting pigeons, major diseases are viral diseases (Liu et al., 2003) and among viral disease, NDV is most important (Ballouh et al., 1985). In pigeons, ND is caused by pigeon paramyxovirus serotype-1 which is a variant of APMV-1 causing disease in poultry and it was first isolated from the Middle East in 1978 (Kaleta et al., 1985). However, it was different from reference NDV strains as it contains unique monoclonal antibody binding profiles (Collins et al., 1989). Different bird species other than commercial chicken may serve as a source of NDV spread to poultry industry (Roy et al., 1998). (Alexander et al., 1984) reported that NDV spread to domestic chickens had occurred in different countries due to fecal contamination of feed by feces of ND infected pigeons. Non-vaccinated birds can get the infection by NDV of pigeon origin as occurred by 2006 case of NDV in Scotland (Dilaveris et al., 2007). Virulent NDV strain from pigeons was isolated in India and showed the ability to induce ND infection in chickens without prior infection (Roy et al., 2000).

In 1981, an epizootic of ND similar disease in show and racing pigeons occurred in Sudan and Italy (Eisa and Omer, 1984) and in next few years, it not only spread throughout Europe but also to North America (Wilson, 1986). Avian paramyxovirus type-1 can also cause disease in pigeons (Mubarak et al., 2001). Only velogenic strains of pigeon type NDV (PPMV-1) cause disease in pigeons (Saif, 2003). ND is an important problem of pigeons in Pakistan and the nervous form of the disease in pigeons is called 'Jholah' in the local language in Pakistan (Arshad, 1984). (Munir et al., 2015) investigated carrier potential of pigeons towards NDV and found 20% of samples positive for the presence of NDV. (Korotetskiĭ et al., 2009) stated that pigeons pose a serious threat to commercial poultry in Russia, Ukraine and Kazakhstan for the spread of NDV. In a recent study, (Shabbir et al., 2012) reported similar isolates originating from pigeons in commercial poultry. (Alexander, 2001) studied virulent field isolates of NDV from 2000 to 2009 in Europe in domesticated pigeons and commercial poultry. These studies showed that epizootic in domesticated pigeons was due to pigeon avian paramyxovirus type 1 and this belongs to a different genetic group 4b (VIb). This group was first reported in Europe in 1981 and still, it continued to cause outbreaks in commercial poultry during the 2000s. This virus strain spread repeatedly to wild birds especially to *Columbidae* family and cause outbreaks in commercial poultry. (Terregino et al., 2003) isolated NDV from Eurasian collard doves and reported them to be one of APMV-1 and similar to pigeon variant group (PPMV-1).

Crested Ibis (*Nipponia nippon*): Also called Japanese crested ibis, is one of the endangered species of the world found in Shaanxi province in China where almost 94% of China's bird species are present (Duan et al., 2014) and also in Japan. Chen et al. (2013) collected two NDV isolates from sick birds in China. He investigated complete phylogenetic analysis and pathogenicity assessment of these isolates. These strains have amino acid sequence ^{112}-R-R-Q-K-R-F-^{117} at protein cleavage site which showed virulent nature of these strains. These isolates were homologous with strains isolated from commercial poultry in the same geographical location from 2006 to 2010.

House sparrow (*Passer domesticus*): House sparrow (*Passer domesticus*) commonly found wild bird in different countries of the world like Pakistan, India and China. Due to his small size, it has an easy contact with poultry birds. A study was done in which five NDV strains were isolated from house sparrows living around the poultry farms in the southern provinces of China. Phylogenetic analysis was done for characterization of these isolates. All NDV isolates

except one were found to be velogenic and virulent for commercial chickens. These isolated strains had amino acid sequence ^{112}R/K-R-Q-K/R-R-F^{117} in protein cleavage site which is specific for velogenic NDV. Phylogenetic analysis indicated that these isolates belong to genotype VII and have close resemblance with strains isolated from routine NDV outbreaks in commercial chickens since 2000. One isolate of NDV from house sparrow belonged to genotype II and was proved to be vaccine strain. The result of this study confirmed that house sparrow carries the virulent NDV strains and the same genotype of viruses that are circulating in commercial poultry are existing in house sparrows living around poultry farm in southern China (Zhu et al., 2010).

Double-crested cormorants (*Phalacrocorax auritus*): The double-crested cormorant is native to North America and is one of six species of cormorants. Several reports suggest the presence of NDV in double-crested cormorants in different countries of the world. In 1990, NDV outbreak in breeding colonies of double-crested cormorants across western Canada caused high mortality of Juvenile double-crested cormorants (Wobeser et al., 1993). In 1992, a widespread epidemic of ND occurred affecting breeding colonies of double-crested cormorants in western Canada and the north-central USA. It was the first time that ND outbreak caused high mortality in wild birds. The exact source and epidemiology of the disease in double-crested cormorants was unknown (Kuiken et al., 1998). (Heckert et al., 1996) supported the evidence of spread of vNDV in 1992 from cormorants to commercial turkey flocks in North Dakota which greatly emphasis on the danger of NDV spread in domestic poultry from wild bird's population.

House crow (*Corvus splendens*): Indian house cow or simply house crow is a native bird of India and its neighboring countries (Ali, 2002). It has spread to a large number of countries where it spread pathogens to domestic bird's population (Cooper, 1996). House crow is considered as serious avian pest and present in close proximity to poultry birds in villages and towns (Naureen, 2001). Different reports indicate the spread of virulent strains of NDV to domestic poultry by house crow. Most studies on crows are confined to Asia (Duggal et al., 1986). During an outbreak of NDV in fowls, Cooper (1931) first observed crow's mortality. (Pearson and McCann, 1975) studied 9446 wild birds in southern California and isolated one vvNDV strain. (Sulochana et al., 1981) isolated NDV from Indian house crow and found 10 birds positive for NDV out of 82 crows studied and reported one strain to be highly pathogenic when given to experimental chicken. (Ibu et al., 2009) reported the involvement of crows in the spread of vNDV to commercial poultry. A report suggests serious threat to spread NDV to commercial poultry industry was posed by crows in Ukraine, Russia and Kazakhstan (Korotetskiĭ et al., 2009). (Munir et al., 2015) isolated NDV from crows living near poultry farms in Punjab province of Pakistan and found 16% samples positive for NDV.

Pheasants (*Phasianus colchicus*): The term indicates a hybrid of ring-necked pheasants. Some species like golden pheasant (*Callonetta leucophyrs*) form a smaller proportion of pheasant family and are named as game birds. Pheasants are reared in different countries around the globe and there is a considerable trade of day-old pheasants internationally (Aldous and Alexander, 2008). Clinical signs reported in pheasants infected with virulent NDV include anorexia, depression, white green watery diarrhea and nervous signs like incoordination and head shaking (Jørgensen et al., 1998) with variable mortality rates. Vaccination against NDV in pheasant is done as routine practice and it only provides protection against disease signs but not from virus replication (Muller et al., 1990). Several publications in the 1940's discussed about the presence of NDV in pheasant population. In 1963, the first outbreak of ND in free-living pheasants in the UK was reported by Beer (1976). (Capua et al., 1994) isolated PPMV-1(responsible for ongoing panzootic in pigeons) in pheasant which were imported by Italy. In 1996, an outbreak of ND hit about a population of 12,000 free-range pheasants in Denmark and was associated with virulent strains of NDV (Jørgensen et al., 1998) and had close resemblance with virus strains isolated from NDV outbreaks in poultry in Scandinavia and British Isles during 1995-1997. Current information suggests that viruses isolated from different pheasant population don't have a genetic resemblance to a particular group and hence can be found in all possible genetic lineages (Alexander et al., 1999). This may confirm the idea that all strains of NDV hit pheasants and mostly they get infected with strains present in domestic poultry.

Peafowl (*Pavo cristatus*): Peafowl is omnivorous belongs to *Phasianidea* family and order *Galliformes*. The male peafowl is known as Peacock. Peafowl is a

wild bird but can be raised in captivity as an ornamental bird (Titilincu et al., 2009). Different reports suggest the presence of NDV in peacock. Not enough data regarding ND outbreaks in Indian peacocks is present except for a report of a natural outbreak in 1968 (Goto et al., 1968). (Dou and Yang, 2007) reported isolation of NDV from peacock in China. (Munir et al., 2012) the reported death of 190 peacocks in Jallo wildlife park, Lahore, Pakistan within one week in an outbreak of ND in 2012. (Mustafa et al., 2015) reported ND endemic in peacocks at Tharparkar desert, Sindh province of Pakistan during 2012 and 2013 where there was about 40,000 estimated peacock population was present.

Waterfowl (*Anatidae*): Waterfowl includes ducks, geese, swan and NDV strains isolated from them are mostly lentogenic causing no apparent disease (Kida et al., 1980). Among avian species, ducks and geese are slightly susceptible to NDV infection as compared to others (Kaleta and Baldauf, 1988). (Takakuwa et al., 1998) conducted a study on migratory waterfowl in Alaska and Serbia for detection of NDV. He sequenced 11 samples and found 5 having a pair of dibasic amino acid sequence at the cleavage site of fusion gene which shows virulent nature. He concluded that virulent NDV strains are maintained in migratory waterfowl in nature which may transmit it to domestic poultry and mutated to pathogenic in chickens. (Zhang et al., 2012) characterized two vNDV strains taken from outbreaks in ducks in China. The amino acid sequence was found virulent and cause 100% mortality when given to experimental chickens. He concluded that virus transmission of vNDV strains may occur between chicken and ducks. (Roy et al., 2000) isolated NDV virus during outbreaks on duck and chicken farms in 1993 and found all of them to be virulent nature. Generally, lentogenic NDV strains that circulate among the waterfowl population have the ability to become pathogenic after replication in domestic chicken. However comparative studies of NDV infection in chicken and waterfowl are rare. (Shengqing et al., 2002) took avirulent NDV strain from waterfowl and passaged it in experimental chickens. After several passages through air sac followed by passages through the brain, the virus becomes highly virulent and caused 100% mortality. He concluded that avirulent NDV strains found in waterfowl population when transmitted to chicken have the potential to become virulent one.

Ostrich (*Struthio camelus*): Commercial rearing of the ostrich is the general practice in Pakistan and its neighboring countries like Iran where it started about 10 years ago (Ghiamirad et al., 2010). The first case of NDV in ostriches was observed in the 1950s in zoo birds of Africa (Alexander, 2001). (Samberg et al., 1989) reported the first outbreak of NDV in commercially rearing ostriches in Israel and observed 28% mortality. (Huchzermeyer and Gerdes, 1993) isolated NDV strains having low mortality signs from three outbreaks in commercial ostriches. (Jørgensen et al., 1998) reported vNDV from ostrich flock in Denmark. Recently, (Ghiamirad et al., 2010) isolated highly virulent NDV strain from a commercial ostrich farm in Iran.

Turkeys (*Meleagris*): Turkeys are highly susceptible to NDV and show similar signs as in chickens. These signs mostly include depression, bloody diarrhea and incoordination (Cattoli et al., 2011). Wakamastu et al. (2006) experimentally infected 6-weeks old commercial Turkey and 3 weeks old SPF chicks with vNDV isolated from California during the outbreak in 2002. All birds become sick and showed the same clinical picture. (Piacenti et al., 2006) used five different NDV isolates to experimentally infected Turkeys to examine their pathogenesis. Birds infected with vNDV strains showed a clinical sign. These studies showed a similarity of strains infecting both domestic poultry and Turkeys and might show the potential of Turkeys to spread the disease to commercial poultry.

vNDV has also been reported in different pet birds including budgerigars (*Melopsittacus undulates*), Conures and Amazon parrots (Cattoli et al., 2011). These birds are often kept in captivity near poultry farms as people are usually unaware of the spread of disease from them. No, fully characterization has made about circulating viruses in these pet birds.

Diagnosis

Despite advances in diagnostic approaches, access to modern diagnostic methods is restricted to only a few laboratories. So in developing countries including Pakistan, still a tentative diagnosis of NDV is made on the basis of the clinical picture, postmortem lesions especially pinpoint hemorrhages in proventriculus and in caecal tonsils. Conventional methods like Haemagglutination inhibition (HI) test and virus isolation are considered standard tests to identify NDV during outbreaks (Shabbir et al., 2012).

Regarding the importance of the poultry industry to Pakistan's economy, Government in collaboration with the private sector should work for the availability of modern diagnostic tools.

Isolation and genotype identification for NDV is compulsory and prescribed test for international trade and is considered a method of choice for confirmatory diagnosis (Cattoli et al., 2011). NDV can be isolated from bird's respiratory and fecal secretions and also from tissues of dead birds like caecal tonsils, proventriculus and intestine. For NDV isolation using embryonated eggs, protocols are adapted as described by OIE. Specific pathogen free (SPF) Embryonated chicken eggs of 9–11 days are suitable for culturing NDV. Due to lack of availability of SPF eggs in Pakistan, embryonated eggs of 9-11 days are used and obtained from commercial flocks having low antibody titer to NDV and to counter maternal antibodies, chorioallantoic sac (CAS) route is preferred over yolk sac. Harvesting of allantoic fluid is done before 15 days of incubation as at this age, absorption of antibodies started from egg yolk if the embryo is alive with virus inoculums (Shabbir et al., 2012). With only a single passage, more than 85% of virus isolations are achieved however less than 10% virus isolations require one blind passage. To make final isolation accelerated, two passages at three-day intervals can be given. Then the allantoic fluid is subjected to haemagglutination assay (HA) to check the presence of the virus. If the HA test is positive, the virus is confirmed through the use of haemagglutination inhibition (HI) test which uses NDV specific antisera (Cattoli et al., 2011).

Laboratory base diagnosis has been improved in Pakistan with the availability of modern molecular diagnostic tools in research institutes and laboratories. The major advantage is a rapid diagnosis of organisms causing the similar symptoms at the same time e.g. multiplex PCR can differentiate organisms in a single test. However technical training is constantly required for use of such sophisticated technology as an evolutionary mechanism of NDV often cause the failure of already established protocols (Munir et al., 2012). Technique based molecular tools are not only used for detection but also for rapid genetic characterization of the virus. Reverse transcriptase polymerase chain reaction (RT-PCR) is the most sensitive technique among different molecular diagnostic tools developed for detection of NDV. Most of research institutes and laboratories in Pakistan use specific primers from already published data in the world for identification of NDV strains. Most of the work related to antigen characterization and sequencing is already being done in collaboration with foreign organizations/ OIE reference lab (Shabbir et al., 2012). Molecular diagnosis of NDV can be done through two methods (1) detection of virus using specific primers against comparatively conserved regions of genome like L, M and NP gene and (2) detection of vNDV using F gene region involving cleavage site and this is difficult because of geographical variations (Hoffmann et al., 2009). Using different freely available software, primers can be designed by taking information from nucleotide data available at NCBI. The student in different Universities can take advantage of this but it requires the attention of Government and research institutes to guide them properly.

Due to lack of cost effective kits, these above mentioned molecular diagnostic tools are not common in the field and limited to research institutes or university laboratories. Despite long debate on their sensitivity, conventional methods are still predominately used for field diagnosis of NDV and HI is the most frequently used test. In collaboration with private poultry sector, the Government could work for the establishment of modern labs having all diagnostic facilities in regions where poultry population is in abundance. This would be helpful for rapid diagnosis and making strategy to avoid spread or possible outbreak of disease.

Current status and economic impact of NDV on poultry industry of Pakistan

In Pakistan, the commercial poultry industry was started in the 1960's and now it is the second bigger industry in Pakistan with an annual growth rate of 8-10% (Hussain et al., 2015). It provides employment to 1.5 million with approximate current investment of 200 billion rupees. Pakistan is the 11th largest poultry producer in the world with an annual production of 1.02 billion broilers (GOP, 2016). Protein is an important part to make human diet balance. Out of two main protein sources, animal proteins have an upper edge over plant protein source (Grigg, 1995) and main animal protein sources include poultry meat, mutton, milk, eggs and beef. Poultry meat has an advantage over red meat as it has less fat and cholesterol percentage as compared to beef and mutton and is at an affordable cost for people of developing countries (Ghafoor et al., 2010). In developing countries like Pakistan, broiler meat is considered as the cheapest

protein source and egg availability is continuously increasing at a rate of 4% annually (Ashraf and Shah, 2014). Pakistan is included in countries where 66% population is deficient in protein diet. World Health Organization (WHO) reported average daily requirement for animal protein as 27g per person, while in Pakistan it is about 17g per head per day. The poultry industry has an important role in reducing the gap between demand and supply for protein. 30-35% of total meat consumption in Pakistan is from poultry products. Pakistan mainly exports live poultry and meat to Afghanistan, Turkey, Iran, Hong Kong, Bahrain and Vietnam. In 2010-11, Pakistan exported 553 Tones of poultry meat having a value of 1.08 billion (PKR) but this decreases to 365 million rupees in 2012 (Hussain et al., 2015). During 2015-16, Pakistan meat exports were of US$ 303.468 million and mostly composed of eggs and white meat (GOP, 2016). Exporting wild birds is an important source of overseas currency profit (Sand, 1997) and its worth in the international market was about eight billion US dollars in 2002. It is estimated that Pakistan exports birds having worth of 100 million rupees. Estimated annual global trade of pet animals is around 350 million animals having worth of US$20 billion. About one quarter is considered illegal without any testing or inspection (Karesh et al., 2007). Pakistan is a big market of importing exotic birds as people keep them as status symbol. Moreover, Himalayan region of China is a hub of illegal wildlife trade (Yi-Ming et al., 2000) which pose great thread to local industry of Pakistan. In Pakistan, non-availability of modern scientific tools for species identification also results in increased illegal wildlife trade (Rehman et al., 2015).

ND is prevalent worldwide and causes annual losses in millions of dollars (Susta et al., 2011). NDV is considered an important constraint for poultry products throughout the world especially in developing countries (Branckaert and Guèye, 1999) as it not only causes high mortality and production losses but also results in economic losses by trade restrictions. NDV is always devastating to the poultry industry and outbreaks are continuously reported even in vaccinated broiler flocks in Pakistan (Siddique et al., 1986). Production losses may result due to a 90% drop in egg production when virulent NDV hit parent layer flock (Sharif et al., 2014). NDV is still endemic mostly in developing countries and has a major impact on villages where people's income largely depends upon poultry farming (Mohamed et al., 2011). vNDV viruses result in 100% mortality and also a considerable drop in egg production (Alexander et al., 1997). Several recent outbreaks in commercial broiler and layer flocks have been reported and phylogenetic analysis of suspected isolates have revealed their virulent nature (Shabbir et al., 2012).

Any outbreak of NDV in breeder flock would be highly fatal as the poultry industry in Pakistan completely depends on day-old GP flocks imported from different countries including Germany, Holland, and the USA. Currently, there is an estimated population of 730,000 GP flock in Pakistan (Mukhtar et al., 2012). Broiler breeders have almost 65 weeks lying cycle in which they lay approximately 199 eggs on an average in which almost 183 eggs are hatchable (Farooq et al., 2014). 183 hatchable eggs mean 183 broilers and if we look at the average price of day-old chick in Pakistan it is about 30 PKR. The death of one breeder bird means not only loss of rearing cost and current price which is estimated around 5000 PKR per bird but also production loss of about 5500 PKR which would be highly devastating for a farmer. Although strict biosecurity measures are adapted to avoid diseases in breeder flocks but seeing disease prevalence and transmission involving wild birds might cause disease in parent flock which would cause unbearable loss to the economy as 1.5 million families are linked to the poultry industry. Recent outbreaks in Pakistan caused huge economic losses to farmers during 2011 and 2012. NDV has killed 45 million chickens at commercial poultry farms resulting estimated loss of 6 billion PKR alone in Punjab. NDV cause death of 190 peacocks during NDV outbreak in Jallo Wildlife Park in Lahore, Punjab province of Pakistan (Hussain et al., 2015). OIE categorize NDV as list 'A' disease and an outbreak of mesogenic or velogenic ND is required to report to OIE which may result in severe trade restrictions by business partner and these trade restrictions will cause huge losses to country's economy. Vaccination and treatment cost of affected flocks also aids in economic losses rendered by NDV. The disease also causes low egg and meat quality which not only affect the economics of farmer but also make this cheap protein source unaffordable for poor people (Sharif et al., 2014).

Conclusion and Recommendations

Newcastle disease is still economically important and poses a great threat to the poultry industry. Often

free-living wild birds or pet birds are associated with outbreaks of NDV in commercial poultry. In the world, vaccination coupled with strict biosecurity is available best option to control the disease. In Pakistan, vaccines are imported instead of using field isolates for vaccine production. These subs-standard imported vaccines don't match with field strains and are ineffective for control of the disease. Farmers are usually illiterate and the concept of biosecurity is limited to some larger farms. People are unaware of the wild bird's importance in the spread of disease. They keep ornamental birds in close proximity to farms and also migratory or resident wild birds have access to commercial poultry. Traditional methods of diagnosis like postmortem signs, haemagglutination assay (HA) should be discouraged as they are not confirmatory. Instead, modern laboratory diagnostic methods should be adapted for rapid detection and characterization of virus strains to limit the disease spread. Field diagnosis coupled with modern molecular diagnostic tools could be used for accurately characterizing risk. Currently, no full characterization of any virus strain circulating in bird's population has been made and limited data is available about circulating strains in wild birds. Due to the evolving nature of the virus, novel strains might exist in resident wild birds or migratory birds could introduce new strains that would be devastating to the industry. The government should consider steps to enhance awareness in farmers or field personnel's as well as the provision of modern diagnostic toolsto laboratories to confront the disease and limit the economic losses. Research institutes should design studies to detect and fully characterize NDV strains from wild birds so that their potential to spread the disease to domestic poultry could be judged.

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Author's Contribution

All authors contribute equally in preparation of manuscript.

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