

Review

Newcastle Disease: Present status and future challenges for developing countries

Ashraf, A¹. and Shah, M. S.^{2*}

¹Department of Wild Life and Fisheries, Government College University, Faisalabad, Pakistan.

²Animal Sciences Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

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Newcastle disease (ND) 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. The virus belongs to family Paramyxoviridae and it has 10 serotypes designated as APMV-1 to APMV-10. The Newcastle disease virus (NDV) is endemic in many countries of the world. The first outbreak of Newcastle disease was observed at Newcastle during 1926. Later, it was found in various parts of the world. NDV spreads mostly by the direct contact between healthy and diseased birds or also by the excretions of infected birds. This disease can vary in nature from mild to severe depending upon the type of the virus. NDV can infect both domestic and wild birds. This disease can have a devastating effect on poultry due to high morbidity and mortality rates. In unvaccinated chickens, the morbidity and mortality rates may reach up to 100% each, depending upon the virulence of the NDV. Live virus vaccines and killed oil based vaccines are used in many countries to prevent the disease in chickens. Despite the extensive use of vaccines, outbreaks are continuously occurring in various parts of the world resulting in huge losses since few years. Moreover, extensive use of vaccines has made the situation favorable for genetic modification of the pathogenic virus. Keeping these issues in mind, future challenges are highlighted in detail.

Key words: Newcastle disease, vaccines, outbreaks, genetic modification, avian paramyxovirus-1.

INTRODUCTION

Newcastle disease (ND) is one of the most important viral diseases (Orsi et al., 2010). It is an acute infectious viral disease of domestic poultry and other species of birds regardless of variation in sex and age (Alexander, 2003; Haque, 2010; Iram et al., 2013). ND causes huge economic losses to the commercial poultry farmers round the world (Aldous et al., 2003; Qin et al., 2008; Diel et al., 2012). Etiological agents of ND are virulent strains of avian paramyxovirus - 1 (Qin et al., 2008; Yu et al., 2001; Choi et al., 2010). The disease is characterized by respiratory, nervous system impairment, gastrointestinal and reproductive problems (Nanthakumar et al., 2000; Tiwari et al., 2004). Newcastle disease is commonly known as Ranikhait disease in India (Narayanan et al., 2010; Ravindra et al., 2009) and also in Pakistan.

Newcastle disease virus (NDV) has a wide host range, including approximately 241 species of 27 orders, out of known 50 orders of birds (Madadger et al., 2013). More commonly affected species include chickens, turkeys, ducks, pigeons, (Zhang et al., 2011) guinea fowl, Japanese quail and many wild birds of all ages (Nanthakumar et al., 2000). The most susceptible avian species to this disease are chickens (Rezaeianzadeh et al., 2011) and also some mammals like humans, cats and dogs. During the last 40 years, paramyxoviruses were isolated from different animals (Miller et al., 2009).

In several developing countries, ND is endemic and has greatest impact on villages where people's livelihood depends upon poultry farming (Mohamed et al., 2011; Rezaeianzadeh et al., 2011). APMV-1 viruses circulating

in poultry flocks are being characterized (Munir et al., 2012). ND is fatal and still top ranked poultry disease. Annual losses caused by this disease worldwide are in millions of dollars (Waheed et al., 2013; Susta et al., 2010).

ND is an economically important disease and also a major threat to poultry industry (Narayanan et al., 2010). According to variation in strains of NDV, the rate of mortality and morbidity in a flock (Haque et al., 2010) varies from 90-100% (Nanthakumar et al., 2000) along with decrease in egg production (Choi et al., 2010).

Due to the severe nature of Newcastle Disease and the related consequences, NDV is included in "LISTED" agents (reportable disease) by Office International des Epizooties (OIE) (Aldous and Alexander, 2001; Boynuokara et al., 2013). Notification is required by OIE of any outbreak of ND (Cao et al., 2013), when it meets certain criteria of virulence (Cattoli et al., 2011; Munir et al., 2012).

EPIDEMIOLOGY

The epizootics of Newcastle Disease in poultry continue to occur in Asia, Africa, Central and South America while in Europe, sporadic epizootics occur (Naveen et al., 2013). ND is reported consistently from all continents of the globe (Munir et al., 2012).

Major panzootics of ND have been recorded from different parts of the world. The very first panzootic started in 1926 in Southeast Asia from Java, Indonesia and in Europe from Newcastle-upon-Tyne, England (Seal et al., 1995; Arifin et al., 2011), and it remained till late 1950s (Qiu et al., 2011). The second panzootic began in Middle East in late 1960s and spread to other countries till 1973.

The third drastic panzootic caused by neurotropic form of NDV, termed pigeon paramyxovirus type 1 virus, appeared in Middle East about in the late 1970s. In 1981, ND reached Europe then spread rapidly throughout the globe (Mase et al., 2002). The latest and fourth pandemic emerged by late 1980s in Far East, South Africa, and Europe (Qiu et al., 2011). A sporadic form of Newcastle Disease exists in Pakistan throughout the year; only a limited number of outbreaks are reported annually (Munir et al., 2012a). In Southeast Asia, it is endemic and a cause of huge economic losses to commercial poultry (Munir et al., 2012 b).

During 2012, severe outbreak of ND occurred in Jallo Wildlife Park in Lahore, Pakistan, caused by APMV 1 serotype. Within a week, it took the lives of approximately 190 peacocks with a 100% mortality rate and 50% loss of the susceptible birds. 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 (Munir et al., 2012c).

ECONOMIC IMPACT

Proteins are a significant part of balanced human diet. There are mainly two proteins sources which are Animals and Plants. In developing countries, human diet is deficient in the animal proteins; approximately 66% population has protein deficient diet (Maqbool, 2002). A single person per day requires 102.7 g protein, while only 69.61 g protein is used by a person per day. The main animal protein sources are mutton, beef, poultry meat, eggs, and milk (Maqbool and Bakhsh, 2007). 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. Availability of egg is increasing at rate of round about 4% annually (Numan et al., 2005).

Poultry production was started as a cottage industry in many developing countries of the world. The production and management for disease control measures were not sufficient because of the lack of scientific knowledge. In Pakistan, approximately 1105.91 million poultry birds are present, from which rural poultry is about 152.44 millions. In village economy, it plays vital role with the contribution of about 3611 million eggs and 100.42 metric tons of the total poultry meat (Khan et al., 2010).

Recent studies by Pakistan Economic Survey (2011-2012) reported that poultry sector generates income and direct and indirect employment for about 1.5 million people till 2012. Its contribution in agriculture is 6.40% and in livestock 11.50%. In total, meat production of country and poultry meat contributes 25.8%. Poultry sector has rapid growth of about 8-10% every year, which shows its inherent potential. According to currently conducted survey, the present investment in the Pakistan poultry industry is about Rs. 200.00 billion.

ND and avian influenza (AI) are major concerns of animal husbandry due to hazardous infections (Ge et al., 2012). All over the world, poultry industry is facing severe economic losses with every passing year (Haque et al., 2010; Khan et al., 2011).

ETIOLOGY

According to taxonomy of virus, NDV belongs to order Mononegavirales, family Paramyxoviridae and subfamily Paramyxovirinae (Cattoli et al., 2011). The subfamily is divided into five genera: Morbillivirus, Respirovirus, Henipavirus, Rubulavirus, and Avulavirus (Miller et al., 2009); all the avian paramyxoviruses APMVs are part of genus Avulavirus. The virus exists in 10 serotypes; APMV-1 to APMV-10 (Waheed et al., 2013), but all NDV isolates belong to serotype 1 (APMV-1). APMV-1 is

synonymous with NDV (Cattoli et al., 2011; Miller et al., 2009). Virions are roughly spherical; 150 nm or more in diameter and filamentous (Catroxo et al., 2011). The genome is about 15.2 kb in length (Cao et al., 2013; Zhang et al., 2012) that codes for six structural and two non-structural proteins (Choi et al., 2010). 'Rule of six' should be followed by genome because it should be of polyhexameric length to replicate rapidly. It encodes for six proteins in 3' to 5' direction; these are Nucleoprotein (NP), Large RNA polymerase (L), Fusion (F), Hemagglutinin Neuraminidase (HN), Matrix (M) and phosphoprotein (P) (Linde et al., 2011; Al-habeeb et al., 2013). The proteins W and V are additionally created within the P gene during transcription of mRNA at editing site by insertion of guanines (Linde et al., 2011; Qiu et al., 2011).

In virus particles, NP is the most abundant protein which provides the NDVs core helical nucleocapsid structure. NP is the main regulator in replication of viral genome (Kho et al., 2004). The genomic RNA is associated with NP, P and L proteins to form RNP complex, which serve as template for RNA synthesis (Kho et al., 2003). NP is found to be highly immunogenic, as it induces antibody responses in chickens (Ahmad-Raus et al., 2009).

During a field study in Pakistan, 5% of the field isolates were reported as velogenic, 55% as mesogenic and 40% as lentogenic (Waheed et al., 2013). For chickens, different strains of NDV have great variation in pathogenicity. On the basis of clinical signs in infected chickens, strains of NDV are grouped in to five pathotypes: 1) Asymptomatic enteric: a form that has sub-clinical enteric infection without clear symptoms; 2) Lentogenic: virus present with the mild respiratory infections; 3) Mesogenic: virus presents with rare nervous and respiratory signs while mortality rate is related with the age of susceptible birds (young birds are more susceptible as compare to adults); 4) Viscerotropic velogenic: virus cause haemorrhagic intestinal lesions it is highly pathogenic; 5) Neurotropic velogenic: virus cause high mortalities followed by respiratory and nervous signs (OIE, 2012).

The NDV isolates are differentiated on the basis of *in-vivo* estimation of pathogenicity (Pham et al., 2005). These *in-vivo* tests are mean death time (MDT) in SPF embryonated eggs of chicken, Intracerebral pathogenicity index (ICPI) in 1 day old SPF chicks, and Intravenous pathogenicity index (IVPI) in six weeks old SPF chicks (Wise et al., 2004; Adi et al., 2009; Mohamed et al., 2011). The MDT classifies ND virus strains into the groups: velogenic (takes less than 60 h to kill); mesogenic (takes from 60 to 90 h to kill); and lentogenic (takes more than 90 h to kill). The ICPI classifies ND virus strains by giving indices scores from 2.0 to 0.0. The maximum score of 2.0 is given to most virulent ND virus strain while lentogenic strains are given score close to 0.0. The IVPI classifies the ND virus strains from lentogenic to velogenic. Lentogenic strains and some meso-

genic strains have IVPI values of 0.0, whereas the maximum IVPI indices for a virulent strain is 3.0 (OIE, 2004).

MOLECULAR BASIS OF PATHOGENICITY

The genome of NDV encodes for six major structural proteins. Viral replication, transcription and translation occur in the cytoplasm of the host cell, while virus particles are assembled in plasma membrane by budding (Zanetti et al., 2003). Important pathogenic marker of NDV exists in F protein (Madadgar et al., 2013). Disulphide linkage is present between F₁ and F₂. These proteins enable the virus to attach to the host cell membrane (Wen et al., 2007). At cleavage site, F₀ protein has two pair of basic amino acids that can be cleaved by the host proteases (Pham et al., 2005). Highly virulent NDV has three or more basic amino acids, which are lysine (K) or arginine (R) present at 113 - 116 residues and phenylalanine (F) at position 117 (OIE, 2012). Cleavage of F₀ protein is due to the presence of these basic amino acids in virulent NDV (Boostani et al., 2013). It has been found that avirulent viruses have ¹¹²G/E-K/R-Q-G/E-R-L¹¹⁷ and virulent viruses have ¹¹²R/K-R-Q-K/R-R-F¹¹⁷ amino acid sequence at cleavage site (Pham et al., 2005). Most of the pathogenic APMV-1 viruses for chicken have sequence ¹¹²R/K-R-Q/K/R-K/R-R¹¹⁶ (Choi et al., 2010). Office of International Epizootics (OIE) accepts F cleavage sequence as determinant of primary virulence (Wise et al., 2004). However, if this cleavage sequence is not found, then an Intra Cerebral Pathogenicity Index (ICPI) is required for determination of the virulence.

TRANSMISSION

NDV can infect more than 240 species of birds and it spreads primarily through direct contact between healthy and infected birds. The disease transmits through droppings and secretions from the nose, mouth and eyes of infected birds. The disease spreads by contaminated water, feed and transport. Airborne transmission of the virus is also an important route of transmission for ND (Li et al., 2009).

Mechanical transfer of infected faeces occurs by rodents, insects, dogs, fleas, or scavenging animals (Ullah et al., 2004). Infection takes place by virus inhalation, ingestion or by contact with conjunctiva. The disease may vary from subclinical with no mortality to severe infection, with 100% mortality.

SIGNS AND SYMPTOMS

Clinical signs are dependent on factors such as the virus strain, host species, age of the host, co-infection with

other micro-organisms, environmental stress, and immune status (Al-Habeeb *et al.*, 2013). In chickens, the general symptoms are loss of appetite, listlessness, abnormal thirst, weakness, drop in egg production, air sacculitis, tracheitis and conjunctivitis. Respiratory signs can include sneezing, gasping for air, nasal discharge and coughing, whereas a clear intestinal symptom is a greenish watery diarrhea. Nervous symptoms may consist of paralysis of wings and/or legs, twisting of head and neck or complete paralysis (Bhaiyat *et al.*, 1994). Layers show drop in egg production and misshapen soft egg shells (Hadipour *et al.*, 2011). In acute and severe cases (like neurotropic velogenic strain), death is very sudden and birds die without showing any clinical signs. Dead birds have hemorrhagic or necrotic lesions in mucosa of intestine, cecal tonsils, proventriculus and gizzard. Swollen kidneys and deposition of urates are also common lesions.

DIAGNOSIS

Rapid and accurate diagnosis of ND outbreak is important because it clinically resembles highly pathogenic avian influenza (AI) (Khan *et al.*, 2010). 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 immune-sorbent assay, plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of the ND virus (Chaka *et al.*, 2013). Now RT-PCR is the most exclusively used method to detect AIVs and NDVs (Liu *et al.*, 2011; Haque *et al.*, 2010; Wakamatsu *et al.*, 2007). 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 ELIZA (Tang *et al.*, 2012; Shahzad *et al.*, 2011). Using modern technologies, new diagnostic techniques are being developed for identification and differentiation of NDV strains (Rezaeianzadeh *et al.*, 2011). Other molecular diagnostic tests like real time PCR and nucleotide sequence analysis are also important in viral disease diagnosis (Shabbir *et al.*, 2012; Shah *et al.*, 2011).

PREVENTION AND CONTROL

Vaccines are being used to control and prevent ND. Currently, many inactivated and live ND vaccines are available around the world (Shim *et al.*, 2011; Xiao *et al.*, 2013). Chickens and turkeys are immunized against Newcastle disease. Live virus vaccines are administered by variety of routes and schedules from hatching till grow-out (Cho *et al.*, 2008). Killed virus oil emulsion vaccines

are administered parentally prior to the onset of egg production. Although proper vaccination protects the birds from clinical disease but it does not prevent virus replication and shedding, which results in a source of infection (Chukwudi *et al.*, 2012).

Therefore, the prophylactic vaccination is not used in developed countries (OIE, 2012). In developing countries, there is wide use of vaccines on commercial flocks (Munir *et al.*, 2012b). Anti NDV antibody titers of flocks are continuously monitored and flocks are revaccinated to maintain the protective antibody titers. The breeders and layers are vaccinated against NDV and oil based vaccines are being used prior to onset of egg production for long term immunity (Nadeem *et al.*, 2004). Anti NDV antibody titers of breeder flock is also important to maintain the anti NDV maternal antibody titers of progeny. These maternal antibodies protect chicks from the disease during the first week of life. In spite of extensive vaccination, outbreaks are continuously occurring (Shabbir *et al.*, 2012). To overcome this problem poultry producers are using different combinations of live and killed vaccines in a flock.

Good biosecurity measures are essential to prevent Newcastle disease in poultry flocks. Commercial flocks should not have any contact with domesticated poultry or wild birds or any pet birds. Workers should avoid contact with birds outside the farm. Biosecurity measures include bird-proof houses, feed and water supplies, minimizing travel on and off the facility, disinfecting vehicles and equipments that enter the farm. Pests such as insects and mice should also be controlled. If possible, employees should shower and change into dedicated clothing prior entry into the poultry farm.

PUBLIC HEALTH

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 (Alexander, 2000). 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 (Nolen, 2003).

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 (Alexander, 2000; OIE, 2012). There is no evidence found to support human to human transmission but the potential for human to bird transmission exists (Alexander, 2000; David and Daniel, 2003).

FUTURE CHALLENGES

The Newcastle disease virus has not been studied for its evolutionary origin among various outbreaks time to time. Most of the research work was focused on immunological properties of the virus rather than the genomic properties. Further, the extensive use of vaccines makes the situation more favorable for genetic modifications in pathogenic strains. Therefore in International interest, it is essential to address these issues by conducting research on the following lines: 1) Isolation and molecular characterization of velogenic strains of NDV; 2) complete genome sequence analysis of different NDV isolates for further studies of epidemiology, vaccinology and evolutionary origin; 3) existing real time PCR assays should be validated and measures should be devised for prevention and control of epidemics in future.

REFERENCES

- Adi AAAM, Astawa NM, Putra KSA, Hayashi Y, Matsumoto Y (2009). Isolation and characterization of a pathogenic newcastle disease virus from a natural case in Indonesia. *J. Vet. Med. Sci.* 72(3):313-319.
- Ahmad-Raus R, Ali AM, Tan WS, Salleh HM, Eshaghib M, Yusoff K (2009). Localization of the antigenic sites of Newcastle disease virus nucleocapsid using a panel of monoclonal antibodies. *J. Res.Vet. Sci.* 86:174-182.
- Aldous EW, Alexander DJ (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *J. Avian Pathol.* 30:117-128.
- Aldous EW, Mynn JK, Banks J, Alexander DJ (2003). A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *J. Avian Pathol.* 32, 239-256.
- Alexander DJ (2000). Newcastle disease and other avian paramyxoviruses. *Rev. Sci. Tech.* 19:443 - 462.
- Alexander DJ (2003). Newcastle disease, other avian paramyxoviruses and pneumovirus infections. *J. Diseases Poultry.* 11:63-99.
- Al-Habeeb MA, Mohamed MHA, Sharawi S (2013). Detection and characterization of Newcastle disease virus in clinical samples using real time RT-PCR and melting curve analysis based on matrix and fusion genes amplification. *Veterinary World* 6(5):239-243.
- Arifin MA, Salim SH, Mel M, Abdul Karim MI, Hassan SS (2011). Optimization of Newcastle Disease Virus Production in T-flask. Proceedings of the 2nd International Conference on Biotechnology Engineering, ICBioE'11 May 17-19, Kuala Lumpur, Malaysia, ISBN: 978-983-42978-3-1.
- Bhaiyat MI, Ochiai K, Itakura C, Islam MA, Kida H (1994). Brain lesions in young broiler chickens naturally infected with a mesogenic strain of Newcastle disease virus. *J. Avian Pathol.* 23(4):693-708.
- Boostani AR, Pourbakhsh SA, Momayez R, Charkhkar S (2013). Molecular characterization and phylogenetic study of Newcastle disease virus isolates from the 2010 to 2011 outbreaks in Shiraz, Iran. *Afr. J. Microbiol. Res.* 7(8):657-660.
- Boynukara B, Gulhan T, Coven F, Kiziroglu I, Durmus A (2013). Determination of Newcastle disease virus among wild bird populations in Lake Van basin, Turkey. *Turkish J. Vet. Anim. Sci.* 37:01-09.
- Cao Y, Gu M, Zhang X, Liu W, Liu X (2013). Complete Genome Sequences of Two Newcastle Disease Virus Strains of Genotype VIII. *J. Genome Announcements* 1(1):01.
- Catroxo MHB, Martins AMCRPF, Petrella S, Curi NA, Melo NA (2011). Research of viral agent in free-living pigeon feces (*Columba livia*) in the City of Sao Paulo, SP, Brazil, for transmission electron microscopy. *Int. J. Morphol.* 29(2):628-635.
- Cattoli G, Susta L, Terregino C, Brown C (2011). Newcastle disease: a review of field recognition and current methods of laboratory detection. *J. Vet. Diagn. Investigation* 23(4):637-656.
- Chaka H, Goutard F, Gil P, Abolnik C, Almeida R, Bisschop SPR, Thompson PN (2013). Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. *Trop. Anim. Health Prod.* 45:705-714.
- Cho S, Kwon H, Kim T, Kim JH, Yoo H, Park MN, Park Y, Kim SJ (2008). Characterization of a Recombinant Newcastle Disease Virus Vaccine Strain. *Clin. Vaccine Immunol.* 15(10):1572-1579.
- Choi KS, Lee EK, Jeon WJ, Kwon JH (2010). Antigenic and immunogenic investigation of the virulence motif of the Newcastle disease virus fusion protein. *J. Vet. Sci.* 11(3):205-211.
- Chukwudi OE, Chukwuemeka ED, Mary U (2012). Newcastle disease virus shedding among healthy commercial chickens and its epidemiological importance. *Pakistan Vet. J.* 32(3):354-356.
- David E, Daniel JK (2003). Zoonosis update: Avian influenza and Newcastle disease. *JAVMA*, 222 (11):1534-1540.
- Diel DJ, Susta L, Garcia SC, Killian ML, Brown CC, Miller PJ, Afonso CL (2012). Complete genome and clinicopathological characterization of a virulent newcastle disease virus isolate from South America. *J. Clin. Microbiol.* 50(2):378-387.
- Ge G, Zheng D, Zhao Y, Li H, Liu W, Sun Q, Li J, Yu S, Zuo Y, Han X, Li L, Lv Y, Wang Y, Liu X, Wang Z (2012). Evaluating viral interference between Influenza virus and Newcastle disease virus using real-time reverse transcription-polymerase chain reaction in chicken eggs. *Virol. J.* 9(128):01-08.
- Hadipour MM, Habibi GH, Golchin P, Hadipourfard MR, Shayanpour N (2011). The Role of Avian Influenza, Newcastle Disease and Infectious Bronchitis Viruses during the Respiratory Disease Outbreak in Commercial Broiler Farms of Iran. *International J. Anim. Vet. Advances* 3(2):69-72.
- Haque MH, Hossain MT, Islam MT, Zinnah MA, Khan MSR, Islam MA (2010). Isolation and Detection of Newcastle disease virus from field outbreaks in Broiler and Layer chickens by Reverse transcription-Polymerase chain reaction. *J. Vet. Med.* 8(2):87-92.
- Iram N, Shah MS, Ismat F, Habib M, Iqbal M, Hasnain SS, Rahman M (2013). Heterologous expression, characterization and evaluation of the matrix protein from Newcastle disease virus as a target for antiviral therapies. *Appl. Microbiol. Biotechnol.* [Epub ahead of print]
- Khan TA, Rue CA, Rehmani SF, Ahmad A, Wasilenko JL, Miller PJ, Afonso CL (2010). Phylogenetic and Biological Characterization of Newcastle Disease Virus Isolates from Pakistan. *J.Clin. Microbiol.* 48(5):1892-1894.
- Kho CL, Tan WS, Tey BT, Yusoff K (2003). Newcastle disease virus nucleocapsid protein: self-assembly and length-determination domains. *J. Gen. Virol.* 84:2163-2168.
- Kho CL, Tan WS, Tey BT, Yusoff K (2004). Regions on nucleocapsid protein of Newcastle disease virus that interact with its phosphoprotein. *Archives Virol.* 149:997-1005.
- Li X, Qiu Y, Yu A, Chai T, Zhang X, Wang JLD, Wang H, Wang Z, Song C (2009). Degenerate primers based RT-PCR for rapid detection and differentiation of airborne chicken Newcastle disease virus in chicken houses. *J. Virol. Methods* 158:1-5.
- Linde AM, Munir M, Zohari S, Stahl K, Baule C, Renstrom L, Berg M (2011). Complete genome characterisation of a Newcastle disease virus isolated during an outbreak in Sweden in 1997. *J. Virus Genes* 41:165-173.
- Liu H, Zhao Y, Zheng D, Lv Y, Zhang W, Xu T, Li J, Wang Z (2011). Multiplex RT-PCR for rapid detection and differentiation of class I and class II Newcastle disease viruses. *J. Virol. Methods* 171:149-155.
- Madadgar O, Karimi V, Nazaktabar A, Kazemimanesh M, Ghafari MM, Dezfouli SM A, Hojjati P (2013). A study of Newcastle disease virus obtained from exotic caged birds in Tehran between 2009 and 2010. *Avian Pathol.* 42(1):27-31.
- Maqbool A (2002). Marketing of commercial poultry, poultry meat and eggs in Faisalabad City. M.Sc. Thesis University of Agriculture Faisalabad, Pakistan.
- Maqbool AA, Bukhsh K (2007). Issues and Economics of poultry production: a case study of Faisalabad, Pakistan. *Pak. Vet. J.* 27(1):25-28.

- Mase M, Imai K, Sanada Y, Sanada N, Yuasa N, Imada T, Tsukamoto K, Yamaguchi, S (2002). Phylogenetic analysis of newcastle disease virus genotypes isolated in Japan. *J. Clin. Microbiol.* 40(10):3826-3830.
- Miller PJ, Estevez C, Yu Q, Suarez DL, King DJ (2009). Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccines formulated with wild type and recombinant viruses. *Avian Dis.* 53:39-49.
- Mohamed HA, Kumar S, Paldurai A, Samal SK (2011). Sequence analysis of fusion protein gene of Newcastle disease virus isolated from outbreaks in Egypt during 2006. *Virology J.* 8(237):01-04.
- Munir M, Abbas M, Khan TA, Zohari S, Berg M (2012a). Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. *Virol. J.* 9(46):01-11.
- Munir M, Shabbir MZ, Yaqub T, Shabbir MAB, Mukhtar N, Khan MR, Berg M (2012). Complete Genome Sequence of a Velogenic Neurotropic Avian Paramyxovirus 1 Isolated from Peacocks (*Pavo cristatus*) in a Wildlife Park in Pakistan. *J. Virol.* 86(23):13113-13114.
- Munir M, Zohari S, Abbas M, Berg M (2012b). Sequencing and analysis of the complete genome of Newcastle disease virus isolated from a commercial poultry farm in 2010. *Archive Virol.* 157, 765-768.
- Munir S, Hussain M, Farooq U, ZaidUllah Jamal Q, Afreen M, Bano K, Khan J, Ayaz, S, Kim KY, Anees M (2012c). Quantification of antibodies against poultry haemagglutinating viruses by haemagglutination inhibition test in Lahore. *Afr. J. Microbiol. Res.* 6(21):4614-4619.
- Nadeem Y, Chaudhary TM, Shah MS, Ashraf A (2004). Oil adjuvanted newcastle disease vaccine production using local viral isolates. Proceedings of 24th Pakistan Congress of Zoology, pp. 51-56.
- Nanthakumar T, Kataria RS, Tiwari AK, Butchaiah G, Kataria JM (2000). Pathotyping of newcastle disease viruses by RT-PCR and restriction enzyme analysis. *J. Vet. Res. Commun.* 24:275-286.
- Narayanan MS, Parthiban M, Sathiya P, Kumanan K (2010). Molecular detection of Newcastle disease virus using Flinders Molecular detection of Newcastle disease virus using Flinders Tehnology Associates-PCR Tehnology Associates-PCR. *J. Veterinarski Arhiv,* 80(1), 51-60.
- Naveen KA, Singh SD, Kataria JM, Barathidasan R, Dhama K (2013). Detection and differentiation of pigeon paramyxovirus serotype-1 (PPMV-1) isolates by RT-PCR and restriction enzyme analysis. *Trop. Anim. Health Prod.* 10:01-06.
- Nolen RS (2003). Emergency declaration: exotic Newcastle disease found in commercial poultry farms. *J. Am. Vet. Med. Assoc.* 222:411.
- Numan M, Zahoor MA, Khan HA, Siddique M (2005). Serological status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts. *Pakistan Vet. J.* 25(2):55-58.
- OIE (2004). Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.15. <http://www.oie.int>.
- OIE (2012). Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.3.14. <http://www.oie.int/international-standard-setting/terrestrial-manual/access-online>.
- Orsi MA, Doretto Jr, L, Camillo SCA, Reischak D, Ribeiro SAM, Ramazzoti A, Mendonça AO, Spilki FR, Buzinaro MG, Ferreira HL, Arns CW (2010). Prevalence of Newcastle disease virus in Broiler chickens (*Gallus gallus*) in Brazil. *Brazilian J. Microbiol.* 41:349-357.
- Pakistan Economic Survey (2011-2012). Government of Pakistan, Economic Advisor's Wing, Chapter 2 Agriculture, 32-33.
- Pham H M, Konnai S, Usui T, Chang KS, Murata S, Mase M, Ohashi K, Onuma M (2005). *Archive Virol.* 150:2429-2438.
- Qin ZM, Tan LT, Xu HY, Ma BC, Wang YL, Yuan XY, Liu WJ (2008). Pathotypical Characterization and Molecular Epidemiology of Newcastle Disease Virus Isolates from Different Hosts in China from 1996 to 2005. *J. Clin. Microbiol.* 46(2):601-611.
- Qiu X, Sun Q, Wu S, Dong L, Hu S, Meng C, Wu Y, Liu X (2011). Entire genome sequence analysis of genotype IX Newcastle disease viruses reveals their early-genotype phylogenetic position and recent-genotype genome size. *Virol. J.* 8:01-11.
- Ravindra PV, Tiwari AK, Sharma B, Chauhan RS (2009). Newcastle disease virus as an oncolytic agent. *Indian J. Med. Res.* 507-513.
- Rezaeiianzadeh G, Dadras H, Safar A, Ali M, Nazemshirazi MH (2011). Serological and molecular study of Newcastle disease virus circulating in village chickens of Fars province, Iran. *J. Vet. Med. Anim. Health* 3(8):105-111.
- Seal BS, King DJ, Bennett JD (1995). Characterization of newcastle disease virus isolates by reverse transcription PCR coupled to direct nucleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis. *J. Clin. Microbiol.* 33(10):2624-2630.
- Shabbir MZ, Goraya MU, Abbas M, Yaqub T, Shabbir MA, Ahmad A, Anees M, Munir, M (2012). Complete Genome Sequencing of a Velogenic Viscerotropic Avian Paramyxovirus 1 Isolated from Pheasants (*Pucrasia macrolopha*) in Lahore, Pak. *J. Virol.* 86(24):13828-13829.
- Shahzad M, Rizvi F, Khan A, Siddique M, Khan MZ, Bukhar SMi (2011). Diagnosis of avian paramyxovirus type-1 infection in chicken by immunofluorescence technique. *Int. J. Agric. Biol.* 13:266-270.
- Shim JB, So HH, Won HH, Mo I (2011). Characterization of avian paramyxovirus type 1 from migratory wild birds in chickens. *J. Avian Pathol.* 40(6):565-572.
- Susta L, Miller PJ, Afonso CL, Brown CC (2010). Clinicopathological Characterization in Poultry of Three Strains of Newcastle Disease Virus Isolated From Recent Outbreaks. *J. Vet. Pathol.* 48(2):349-360.
- Tang Q, Wang J, Bao J, Sun H, Sun Y, Liu J, Pu J (2012). A multiplex RT-PCR assay for detection and differentiation of avian H3, H5, and H9 subtype influenza viruses and Newcastle disease viruses. *J. Virol. Methods* 181:164-169.
- Thomazelli L M, Araujo JD, Ferreira CS, Hurtado R, Oliveira DB, Ometto T, Golono M, Sanfilippo L Demetrio C, Figueiredo ML, Durigon EL (2012). Molecular Surveillance of the Newcastle Disease Virus in Domestic and Wild Birds on the North Eastern Coast and Amazon Biome of Brazil. *Brazilian J. Poult. Sci.* 14(1):01-07.
- Tiwari AK, Kataria RS, Nanthakumar T, Dash BB, Desai G (2004). Differential detection of Newcastle disease virus strains by degenerate primers based RT-PCR. *J. Comp. Immunol. Microbiol. Infectious Dis.* 27:163-169.
- Ullah S, Ashfaq M, Rahman SU, Akhtar M, Rehman A (2004). Newcastle disease virus in the intestinal contents of broilers and layers. *Pak. Vet. J.* 24(1):28-30.
- Waheed U, Siddique M, Arshad M, Ali M, Saeed A (2013). Preparation of newcastle disease vaccine from VG/GA strain and its evaluation in commercial broiler chicks. *Pak. J. Zool.* 45(2):339-344.
- Wakamatsu N, King DJ, Seal BS, Brown CC (2007). Detection of Newcastle disease virus RNA by reverse transcription polymerase chain reaction using formalin fixed, paraffin-embedded tissue and comparison with immunohistochemistry and in situ hybridization. *J. Vet. Diagn. Investigation* 19:396-400.
- Wen M, Chen, ZT, Zhang DX, Yang JL, Zhou BJ (2007). Cloning and sequence analysis of F gene of Newcastle disease virus isolated from Guizhou province, China. *J. Food Agric. Environ.* 10(3&4):484-486.
- Wise MG, Suarez DL, Seal BS, Pedersen JC, Senne DA, King JK, Kapczynski DR, Spackman E (2004). Development of a Real-Time Reverse-Transcription PCR for Detection of Newcastle Disease Virus RNA in Clinical Samples. *J. Clin. Microbiol.* 42(1):329-338.
- Xiao S, Paldurai A, Nayak B, Mirande A, Collins PL, Samal SK (2013). Complete genome sequence of a highly virulent Newcastle disease virus currently circulating in Mexico. *J. Genome Announcements* 1(1):01-02.
- Yu L, Wang Z, Jiang Y, Chang L, Kwang J (2001). Characterization of Newly Emerging Newcastle Disease Virus Isolates from the People's Republic of China and Taiwan. *J. Clin. Microbiol.* 39(10):3512-3519.
- Zanetti F, Rodriguez M, King DJ, Capua I, Carrillo E, Seal BS, Berinstein A (2003) Matrix protein gene sequence analysis of avian paramyxovirus 1 isolates obtained from pigeons. *Virus Genes* 26:199-206
- Zhang S, Wang X, Zhao C, Liu D, Hu Y, Zhao J, Zhang G (2011). Phylogenetic and pathotypical analysis of two virulent newcastle disease viruses isolated from domestic ducks in China. *J. PLoS ONE* 6(9):1-9.
- Zhang Y, Zhang S, Wang X, Zhang G (2012). Complete genome sequence of a subgenotype vii d newcastle disease virus circulating predominantly in chickens in China. *J. Virol.* 86 (24):13849-13850.