Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

Check for updates

ROOTS PRESS

Research Article

Isolation of newcastle disease virus from commercial broilers in district Abbottabad, Khyber Pakhtunkhwa, Pakistan

Najmus Saba[1], Nayyer Yousaf[1], Omer Dad[2], Naqash Khalid[3] and Anita Bano[1]
[1]*Zoology Department of Govt. Postgraduate College for Women Mandian, Abbottabad.*
[2] *Department of Zoology, Hazara University, Mansehra, KPK.*
[3] *Poultry Research Institute Jaba Mansehra, KPK.*

ABSTRACT

The present study was carried out to isolate Newcastle disease virus in commercial broilers in district Abbottabad, KPK, Pakistan from January to June 2023. A total 60 broilers were swab sampled (cloacal and tracheal swabs) i.e. 120 swab samples were collected. The reverse transcription polymerase chain reaction procedure was done for isolation of Newcastle disease virus in National Reference Laboratory for Poultry Disease, Islamabad. Results revealed that out of total samples, only 5 tested positive for the Newcastle disease virus, indicating a prevalence percentage of 8.33%. Among the different areas surveyed, Qalandar Abad exhibited the highest occurrence of the Newcastle disease virus, with a prevalence rate of 20%. These findings highlight the geographical disparity in Newcastle disease virus prevalence and imply potential variations in risk factors or biosecurity measures among different regions within the district.

Keywords: Newcastle disease virus; commercial broilers; mortality; reverse transcription PCR; paramyxovirus; district Abbottabad.

OPEN ACCESS

Correspondence
Omer Dad
Omerdad173@gmail.com

Article History
Received: November 11, 2023
Accepted: December 10, 2023
Published: December 28, 2023

INTRODUCTION

Poultry

The term "poultry" refers to all birds that are raised or held in captivity for the purpose of producing meat or eggs for human use, other products, refilling supplies of game birds or for any breeding program intended to produce these types of birds (Alexander, 2011). Poultry is the fast-expanding livestock sector in developing nations (Delgado 2005). The most popular white meat is poultry, particularly chicken. Consumption will rise over the next ten years because it provides a rapid source of revenue. Poultry has assumed a significant role in domestic endeavors (Wu, D et al., 2022).

Commercial poultry

The commercial poultry practice is about 40 years old in South Asia (Jin et al., 2017). The country's first commercial hatchery was constructed in Karachi by Pakistan International Airlines (PIA) in the middle of the 1960s (Hussain et al., 2015). With its rapid growth rate of 8–10% per year and yearly output of 1.02 billion broilers, Pakistan is now the 11th largest poultry producer in the world (Mukhtar et al., 2012). Commercially reared birds are susceptible to environmental pathogen exposure (Sharma et al., 1999). In the chicken industry, acute respiratory tract infections are crucial. Infectious bronchitis virus (IBV), Newcastle disease virus (NDV), avian pneumovirus (APV), and Mycoplasma gallisepticum (MG) have all been identified as the most significant pathogens in chicken (Roussan et al., 2008).

Newcastle Disease

Newcastle disease is a highly infectious viral disease that threatens the chicken business globally (Mase et al., 2022). Newcastle disease virus (NDV) sometimes referred to as avian paramyxovirus serotype-1 (APMV-1), is the agent that causes Newcastle disease. It is identified by infection of the neurological, respiratory, and digestive systems. (Ashraf et al., 2016). When Newcastle disease initially occurred in India, it was known as Ranikhet sickness. Toward the start of the 20th century, ND was associated with the introduction of extensive commercial chicken rearing. It is an endemic disease in many impoverished nations and a global hazard. (Ganar et al., 2014).

HISTORY AND EPIDEMIOLOGY

Worldwide Scenario

In 1926, Indonesia and New Castle upon Tyne, England, respectively, received the first reports of Newcastle disease. Multiple ND outbreaks have been reported worldwide, including in Korea, Japan, Australia, India, Sri Lanka, and the Philippines. In Bangladesh, outbreak of ND was published in 1981 (Nooruzzaman et al., 2022). From 1932 to 1998, Australia was free of NDV outbreaks (Ganar et al., 2014). NDV isolates recovered from China and Taiwan between 1996 and 2000 (Yu et al., 2001) and from 1998 to 2002, New South Wales reported a number of outbreaks (Ganar et al., 2014). In the United States, a detectable epidemic of the virulent NDV first appeared in illegally imported game birds in 2002-2003 (Brown et al., 2017). Birds from the Iranian commercial broiler farms around Ahvaz that were vaccinated in 2013–2014 had a significant mortality rate (Boroomand et al., 2016). The first virulent NDV outbreak in the Northeast India during 2011-15 (Nath et al., 2016) and it is isolated from Afghanistan in 2022 (Kabir et al., 2022).

Occurrence in Pakistan

A developing country, Pakistan, totally depends on its industries to stable its economy. The poultry industry is the backbone of its economy, the 2nd largest industry. NDV outbreaks cause huge loss in the economy. (Rehan et al., 2019). Since its discovery in 1963, ND has often been seen in both commercial and backyard chicken flocks in Pakistan (Wajid et al., 2017). The Sindh province's poultry sector productivity significantly decreased in the early 1980s because of the diseases outbreaks (Hussain et al., 2015). In Pakistan, outbreaks of Newcastle disease between 2010 and 2012 were more common in Punjab (Shabbir et al., 2013), led farmers to suffer severe economic losses by killed 45 million chickens (Rehan et al., 2019), as isolated from both commercial poultry and backyard poultry flocks (Abbas et al., 2014). Recently in 2018 NDV isolated from Mansehra Khyber Pakhtunkhwa (Khurshid et al., 2018), also isolated from recent outbreaks during 2019 in district Okara, Punjab (Mehmood et al., 2019).

Strains

Globally, three different types of The Newcastle disease virus (strains with different genome sizes have been identified. The isolates possessed an estimated length of 15,186. The isolates 15,192 nucleotides long from China, and 15,198 nucleotides long in the avirulent strain from Germany (Ganar et al., 2014). It possesses a glycoprotein/lipid membrane and a single-stranded, negative-sense RNA genome (Alexander, D. J. 2011). The virus is naturally quite stable even at low temperatures and a wide range of pH levels, although it has been shown that the NDV becomes unstable around 56 °C. Detergents, lipid solvents, formaldehyde, and oxidizing agents all trigger the sensitivity of NDV (Ganar et al., 2014).

Structure of NDV

NDV is a pleomorphic enveloped virus that measures 200–300 nm in diameter. Its non-segmented, negative-sense, single-stranded genome is about 15 kb long and contains the genetic material for six structural proteins, including the hemagglutinin-neuraminidase (HN), matrix protein, fusion protein, and nucleocapsid protein (NP), as well as two additional, non-structural proteins (W and V) which are also produced inside the P gene during mRNA transcription at an editing site via guanine insertion (Afzal et al., 2015).

Taxonomy and Classification

In the genus Avulavirus, subfamily Paramyxovirinae, family Paramyxoviridae, order Mononegavirales, the ND virus (NDV), also known as Avian Paramyxovirus type 1 (APMV-1), is categorized. Based on tests for neuraminidase inhibition (NI) and haemagglutination inhibition (HI), the genus Avulavirus is classified into nine serotypes. NDV is an avian paramyxovirus of serotype 1 (Alexander, 2011). Newcastle disease virus isolates were classified into four main pathotypes, A virulent NDV, lentogenic NDV, mesogenic NDV and velogenic NDV. Its strains are divided into two classes, With the exception of one strain, chicken/Ireland/1990, all documented class I viruses are low virulent and are generally isolated from wild birds. With the exception of the highly virulent NDV that was responsible for the ND epidemic in 1998 in Australia, the 16 genotypes of class II viruses, such as genotype I NDV, are all low virulent.

Genotype II NDV include low pathogenic strains, some of which are employed as NDV vaccines, and highly virulent strains that are infrequently found. Genotypes III–IX, all strains are virulent. Low virulence genotype X strains have been recovered from different types of poultry, although they are more frequently seen in wild birds. Genotype XI–XVI NDV all strains are virulent (Kapczynski et al., 2013).

Host Range

NDV may infect a wide range of bird species, although the virulence of the virus varies depending on the species. Most poultry species are vulnerable to NDV (Wajid et al., 2017). Numerous poultry species, including broiler, layer chickens, pigeons, ducks, turkeys, ostriches, peacocks, pheasants, double yellow headed parrots, waterfowls, and psittacine are infected with different NDV strains of the virus worldwide (Siddique et al., 2015).

Clinical Symptoms

The clinical manifestations of ND may differ depending on the viral pathotype. Vasogenic (very virulent), mesogenic (moderately virulent), lentogenic (low 10 virulence), and avirulent pathotypes are the four major pathotypes. Vasogenic viruses are associated with significant mortality rates in sensitive birds, which can exceed 100% and are characterized by hemorrhages on the viscera of infected chickens, with viscerotropic viruses causing severe depression and diarrhea. Neurotropic viruses induce neurological symptoms such as ataxia, head tremors and paresis as well as respiratory difficulties. Monogenic viruses typically cause respiratory illness and can be fatal in young birds. Lentogenic viruses cause few clinical symptoms but when they do, they are mainly respiratory in origin. In general, avirulent viruses do not produce any clinical symptoms (Ali et al., 2022).

Life Cycle

Life cycle begins when NDV assaults respiratory epithelial cells, by attaching to substances containing sialic acid, such as gangliosides and N glycoprotein receptors. NDV infection takes place predominantly through a pH-independent process in which the viral envelope merges with the host cell membrane. Infection can also occur by receptor-mediated endocytosis and in rare cases, caveolae-dependent endocytosis. The negative sense RNA genome enters the host cell cytoplasm and transcribes into positive sense mRNA, which eventually translates into viral proteins. The host cell's machinery is used to translate and fold the proteins. The negative sense genomic RNA is transformed to a positive sense anti-genome template for the synthesis of a new negative sense RNA genome once a threshold of the first transcribed N mRNA is met. After being wrapped in the N, P, and L proteins, the newly synthesized genomic RNA is next combined with the matrix and surface glycoproteins to form the nucleocapsid, which is subsequently released from the host cell and the cycle is repeated (Ganar et al., 2014).

MATERIALS AND METHODS

Study Area and Duration

The Abbottabad district of Khyber Pakhtunkhwa, Pakistan, served as the site for the current study.

Samples size

A total of 120 swab samples both tracheal and cloacal from 60 live commercial broilers.

Sampling procedure

Sampling was done from infected commercial live broilers, showing symptoms of Newcastle disease. For collection of samples, wearing gloves and each selected chicken was examined physically for any sign of Newcastle disease virus infection. The Chicken’s mouth was held open, cotton-tipped sterile swab sticks were carefully introduced into the mouth till trachea and gently rubbed the swab clockwise and anticlockwise along the mucosal lining of trachea. In this way sampling was done from trachea. Similarly, samples were collected from cloaca, by carefully grabbing the chicken, exposed the cloacal region, and gently introduce the cotton-tipped sterile swab into cloaca, applying gentle rub the swab clockwise and anti-clockwise, pressure was applied against mucosal lining then swab having collected mucosal secretion was removed. Collected samples were dipped in sterilized swab collecting tube containing BHI media. Samples were labeled according to the information of the shop and area along the sample type with permanent marker, procedure shown in Figures 1-5 (Orsi et al., 2010).

NDV isolation

NDV was identified and confirmed by rapid test RT-PCR according to procedure given in (Siddique et al., 2008) at NRLPD Islamabad. Samples were sent through poultry research institute Jaba for isolation of NDV. By using RT-PCR, the NDV genome was amplified. Master RT Kit (Eppendorf TM Germany) was used in a one-step RT-PCR technique to diagnose samples. The reaction mixture was prepared using the NDV matrix gene, gene-specific primers (5'-TACACCTCATCCCAGACAGG-3' and 5'-AGTCGGAGGATGTTGGCAGC-3') product size 320bp and other reagents

from the Kit (Reaction buffer, dNTPs, RNase inhibitors, reverse and forward primers, MgCl2, Template RNA, Water RNase free, Enzyme mixture) are placed in a thermal cycler (MacMaster, Eppendorf, Germany).

Table 1. Selected areas with number and type of samples.

Selected **A**reas	No. of Birds	Types of Samples	Total No. of Samples
Lower Tanawal	10	10 tracheal, 10 cloacal	20
Qalandarabad	10	10 tracheal, 10 cloacal	20
Mandian	10	10 tracheal, 10 cloacal	20
Supply	10	10 tracheal, 10 cloacal	20
Nawanshahr	10	10 tracheal, 10 cloacal	20
Havelian	10	10 tracheal, 10 cloacal	20
Total	60		120

Figure 1.Tracheal sampling**.**

Figure 2. Cloacal sampling.

Figure 3. Collected samples kept in BHI media.

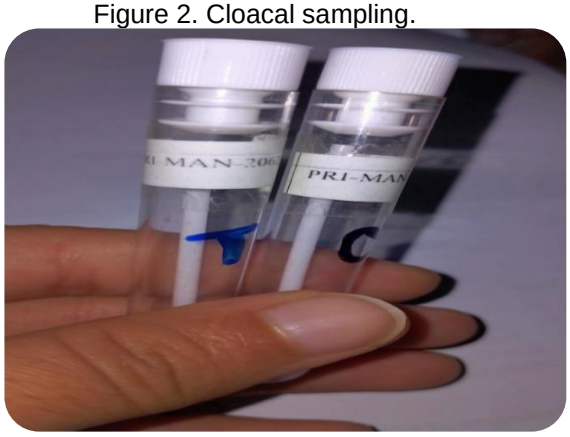

Figure 4. Both cloacal and tracheal samples were tagged.

Statistical Analysis

The Chi-square Test was used SPSS v29.0 software for statistical analysis.

RESULTS AND DISCUSSION

Total Prevalence of NDV

Newcastle disease, one of the most dreadful diseases, affects chicken populations worldwide including Pakistan. It is one of the leading causes of mortality in the poultry business. The pathogen responsible for this deadly illness is avian paramyxovirus type 1 (APMV-1). Three hundred ND outbreaks between 2011 and 2013 were noted. In Pakistan, where the illness is prevalent, sporadic cases of it are regularly reported in wild captive birds as well as commercial and rural poultry (Siddique et al., 2015). The present study revealed that out of a total of 60 broilers sampled, 5 broilers tested positive for NDV. The positive results were observed in different locations showing a prevalence percentage of 8.33%. Specifically, 2 broilers from Qalandar Abad tested positive, 1 broiler from Mandian showed a positive result, 1 broiler

from Supply exhibited a positive test, and 1 broiler from Nawanshahr was also positive. However, it is noteworthy that there were no instances of isolation in Tehsil Havelian and Tehsil Lower Tanawal, indicating that no positive cases were found in those areas among the sampled broilers. This suggests a potential variation in the prevalence or spread of the condition across different locations within the region or the effectiveness of preventive measures taken in those tehsils. The descriptions of the isolates from samples are presented in Table (3) and Figure (6).

Figure 5. samples kept in thermostatic shipment box containing ice cubes and ice pads.

Comparison Among Surveyed Areas

According to the present study, Among the different areas surveyed, Qalandar Abad exhibited the highest occurrence of the NDV, with a prevalence rate of 20%. This indicates that one-fifth of the broiler population in Qalandar Abad was affected by the virus. On the other hand, both Havelian and Lower Tanawal displayed the lowest occurrence of NDV, with no positive samples identified in either area, shown in figure 7. A cross-sectional survey was performed to detect the presence of Newcastle disease virus (NDV) in commercial flocks and backyard poultry, in Kerala, India. A total 2079 samples tested, 167 (8.0%) were positive for the NDV M-gene by RT-PCR (Ravishankar et al., 2022). This survey result is similar to the result of the present study conducted in district Abbottabad showing prevalence percentage of 8.3% of 5 samples positive from total 60 samples collected from commercial broilers.

Table 1. Area wise and total prevalence mentioned.

Selected Areas	Total Birds Sampled	Positive Birds	Negative	Prevalence %	P-value
Lower Tanawal	10	0	10	0%	
Nawanshahr	10	1	9	10%	
Qalandarabad	10	2	8	20%	
Tehsil Havelian	10	0	10	0%	P>0.05
Supply	10	1	9	10%	
Mandian	10	1	9	10%	
Total	60	5	55	8.33%	

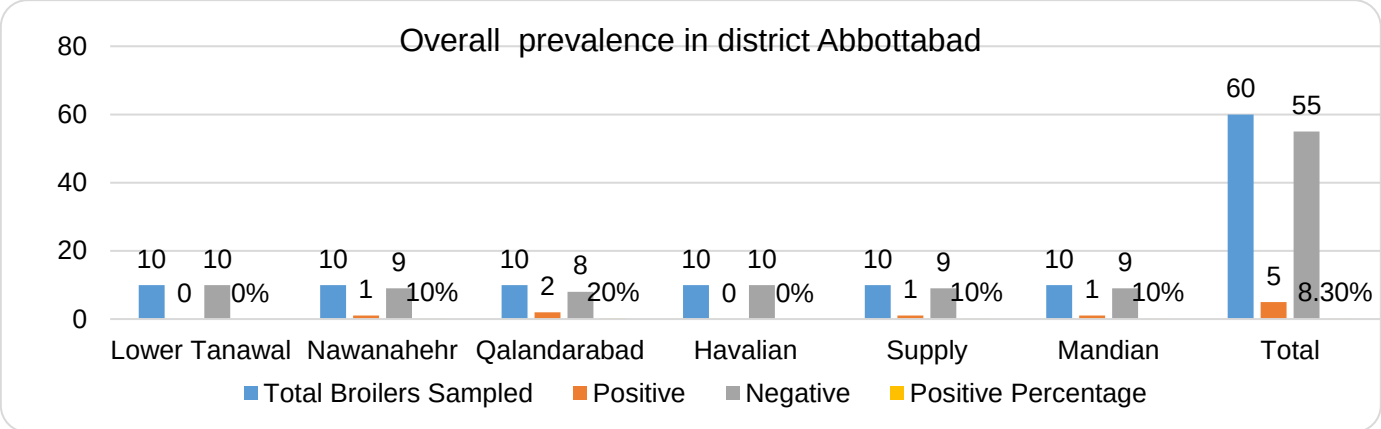

Figure 6. Area wise and total prevalence in study area.

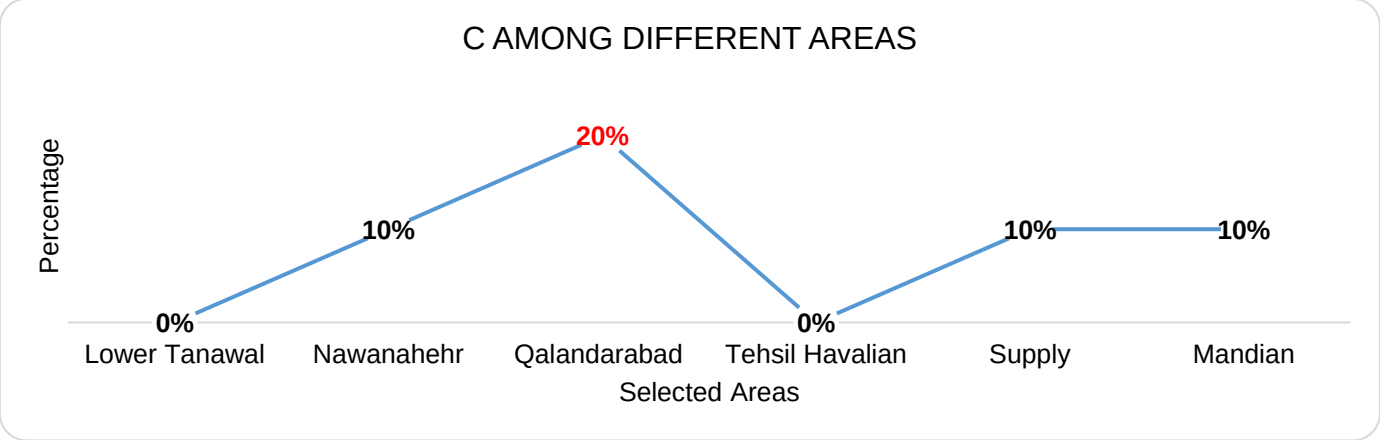

Figure 7. Shows NDV prevalence comparison among selected areas.

CONCLUSION

The present study revealed 8.3% prevalence of NDV in study area. To mitigate the effect of NDV, prevention measures should apply in poultry population to decrease outbreak chances of NDV. Further investigation on NDV should be conducted with the collaboration of experienced researchers in the study field to gain additional insights and specific guidance on a higher scale. Other commercial breeds like commercial breeders and commercial layers should also be considered in study and should select a suitable cell culture for NDV isolation. Should strictly maintain biosecurity measures throughout the study to prevent accidental release of the virus.

ACKNOWLEDGEMENT

The Authors are grateful to Poultry Research Institute Jaba Mansehra KPK and National Reference Laboratory for Poultry Disease (NRLPD) for their help in isolation of NDV through RT-PCR.

CONFLICT OF INTEREST

There is no objection from authors.

REFERENCES

Abbas, M., Shabbir, M. Z., Khaliq, S. A., Ali, S., & Munir, M. (2014). Sequence analysis of the hemagglutinin neuraminidase gene of Newcastle disease virus from Punjab Pakistan. *Advances in Animal and Veterinary Sciences*, 2, 242-247.

Afzal, F., Saliha, U., Fawad, N., Ahmed, S., Munawar, J., Naheed, G., & Siddique, B. (2015). Isolation, characterization of newcastle disease virus and comparitive efficacy of different vaccine regimes in broiler birds. JAPS: *Journal of Animal & Plant Sciences*, 25(4).

Alexander, D. J. (2011). Newcastle disease in the European Union 2000 to 2009. *Avian Pathology*, 40(6), 547-558.

Ali, A., Abdallah, F., Farag, G. K., & Sameh, K. (2022). A Mini-Review on Newcastle Disease Virus in Egypt, With Particular References to Common Vaccines and their Development. *Zagazig Veterinary Journal*, 50(1), 19-36.

Ashraf, A., Shah, M. S. U. D., Habib, M., Hussain, M., Mahboob, S., & Al-Ghanim, K. (2016). Isolation, identification and molecular characterization of highly pathogenic Newcastle disease virus from field outbreaks. Brazilian *Archives of Biology and Technology*, 59.

Boroomand, Z., Jafari, R. A., & Mayahi, M. (2016). Molecular characterization and phylogenetic study of the fusion genes of Newcastle disease virus from the recent outbreaks in Ahvaz,Iran. *Virus Disease*, 27, 102-105.

Brown, V. R., & Bevins, S. N. (2017). A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. *Veterinary Research*, 48,1-15.

Delgado, C. (2005). Rising demand for meat and milk in developing countries: implications for grasslands-based livestock production. *Grassland: a global resource*, 2939.

Ganar, K., Das, M., Sinha, S., & Kumar, S. (2014). Newcastle disease virus: current status and our understanding. *Virus Research*, 184, 71-81.

Hussain, J., Rabbani, I., Aslam, S., & Ahmad, H. A. (2015). An overview of poultry industry in Pakistan. *World's Poultry Science Journal*, 71(4), 689-700.

Jin, S., Jayasena, D. D., Jo, C., & Lee, J. H. (2017). The breeding history and commercial development of the Korean native chicken. *World's Poultry Science Journal*, 73(1), 163-174.

Kabir, H., Hakim, H., Alizada, M. N., Hasan, A., Miyaoka, Y., Yamaguchi, M., ... & Takehara, K. (2022). Isolation, Identification, and Molecular Characterization of Newcastle Disease Virus from Field Outbreaks in Chickens in Afghanistan. *Avian Diseases*, 66(2), 176-180.

Kapczynski, D. R., Afonso, C. L., & Miller, P. J. (2013). Immune responses of poultry to Newcastle disease virus. *Developmental & Comparative Immunology*, 41(3), 447-453.

Khurshid, S., & Rahman, I. U. (2018). Serological status of newcastle disease virus in live broiler birds of Mansehra Khyberpakhtunkhwa, Pakistan. *Adv Biotech Micro*, 11, 555810.

Mase, M. (2022). Hemagglutinin-neuraminidase gene of genotype VII Newcastle disease virus strains isolated in Japan. *Journal of Veterinary Medical Science*, 84(1), 1-5.

Mehmood, A., Rasool, M. H., Anjum, A. A., Zahoor, M. A., & Shafique, M. (2019). Pathotyping and Genetic Characterization of a Highly Virulent Newcastle Disease Virus Strain Isolated from Recent Outbreak in District Okara, Pakistan. *Pakistan Veterinary Journal*, 39(3).

Mukhtar, N., Khan, S. H., & Khan, R. N. A. (2012). Structural profile and emerging constraints of developing poultry meat industry in Pakistan. *World's Poultry Science Journal*, 68(4),749-757.

Nath, B., Barman, N. N., & Kumar, S. (2016). Molecular characterization of Newcastle disease virus strains isolated from different outbreaks in Northeast India during 2014-15. *Microbial Pathogenesis*, 91, 85-91.

Nooruzzaman, M., Hossain, I., Begum, J. A., Moula, M., Khaled, S. A., Parvin, R., ... & Dimitrov, K. M. (2022). The First Report of a Virulent Newcastle Disease Virus of Genotype VII. 2 Causing Outbreaks in Chickens in Bangladesh. *Viruses, 14*(12), 2627.

Orsi, M. A., Doretto Jr, L., Camillo, S. C. A., Reischak, D., Ribeiro, S. A. M., Ramazzoti, A., ... & Arns, C. W. (2010). Prevalence of Newcastle disease virus in broiler chickens (Gallus gallus) in Brazil. *Brazilian Journal of Microbiology*, 41, 349-357.

Ravishankar, C., Ravindran, R., John, A. A., Divakar, N., Chandy, G., Joshi, V., ... & Goyal, S.M. (2022). Detection of Newcastle disease virus and assessment of associated relative risk in backyard and commercial poultry in Kerala, India. *Veterinary Medicine and Science*, 8(3), 1146-1156.

Rehan, M., Aslam, A., Khan, M. R., Abid, M., Hussain, S., Amber, J., ... & Hussain, A. (2019). Potential economic impact of Newcastle disease virus isolated from wild birds on commercial poultry industry of Pakistan: A review. Hosts *Viruses, 6*(1), 1-15.

Roussan, D. A., Haddad, R., & Khawaldeh, G. (2008). Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan. *Poultry Science*, 87(3), 444-448.

Shabbir, M. Z., Zohari, S., Yaqub, T., Nazir, J., Shabbir, M. A. B., Mukhtar, N., ... & Munir, M. (2013). Genetic diversity of Newcastle disease virus in Pakistan: a countrywide perspective. *Virology Journal*, 10, 1-10.

Sharma, J. M. (1999). Introduction to poultry vaccines and immunity. *Advances in veterinary medicine, 41*, 481-494.

Siddique, F. (2015). Preparation and field evaluation of cell culture adapted lyophilized thermostable Newcastle disease vaccine (Doctoral dissertation, University of Agriculture Faisalabad, Pakistan).

Wajid, A., Dimitrov, K. M., Wasim, M., Rehmani, S. F., Basharat, A., Bibi, T., ... & Afonso, C. L. (2017). Repeated isolation of virulent Newcastle disease viruses in poultry and captive non-poultry avian species in Pakistan from 2011 to 2016. *Preventive Veterinary Medicine*, 142, 1-6.

Wu, D., Cui, D., Zhou, M., & Ying, Y. (2022). Information perception in modern poultry farming: A review. *Computers and Electronics in Agriculture*, 199, 107131.

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. *Journal of Clinical Microbiology*, 39(10), 3512-3519.