ECRONICON OPEN ACCESS

EC VETERINARY SCIENCE
Review Article

Epidemiological Status of Newcastle Disease Virus in Pakistan and Susceptible Host Range Around the Globe

Muhammad Farhan Qadir*

Department of College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, China

***Corresponding Author**: Muhammad Farhan Qadir, Department of College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, China.

Received: September 12, 2019; **Published:** October 17, 2019

Abstract

Poultry industry is affected by lot of infectious diseases in which Newcastle disease (ND) is a continuous threat and known as alarming disease for the poultry farmers throughout the world. It is highly contagious disease of poultry birds and also known as Rani Khait disease. ND is caused by avian paramyxovirus serotype-1 (APMV-1) virus. It is also a top List disease of the Office International des Epizooties. Current study will explain the epidemiological status of ND in Pakistan and its susceptible host range with geographical distribution. It is known as respiratory disease including many respiratory signs with nervous signs, diarrhea, complete or incomplete drop of egg production. Mortality is not constant but may reach up to 100%. Disease is transmitted through direct contact with diseased or carrier birds. Infected birds shed virus in the feces as well. Transmission can also be possible through respiratory secretions, contaminated feed, equipment, water or feces. ND virus can survive for long period (many weeks) in the environment. There are several diagnostic tools to detect ND, including serological and molecular methods. It can cause mild conjunctivitis in human and is known as minor zoonotic disease as well. Moreover, regular surveillance and monitoring of birds is mandatory to make authentic control strategies against ND.

Keywords: *Newcastle Disease; ND; Virus; Poultry; Epidemiology; Host; Chickens; Pakistan*

Abbreviations

ND: Newcastle Disease; NDV: Newcastle Disease Virus; vNDV: Virulent Newcastle Disease Virus; APMV: Avian Paramyxovirus; VVND: Viscerotropic Velogenic Newcastle Disease; NVND: Neurotropic Velogenic Newcastle Disease; PVND: Pneumotropic Velogenic Newcastle Disease; WHO: World Health Organization; PCR: Polymerase Chain Reaction; HI: Haemagglutination Inhibition; ELISA: Enzyme linked Immunosorbent Assay; ICPI: Intra-Cerebral Pathogenicity Index; IVPI: Intra-Vertebral Pathogenicity Index; MDT: Mean Death Time

Introduction

Poultry industry is essential form of business and of vital importance in the developing countries. The total investment in poultry sector in Pakistan is almost one billion US dollars. The cheapest source of animal protein is the broiler meat and egg production is increasing 4% per annual. Almost every family in rural area and every 5th family in urban areas is linked with poultry sector in one or the other way [1].

Newcastle disease (ND) is an infectious viral disease of poultry industry, characterized by respiratory excretions, nervous signs, enteric and reproductive infections. ND was first time reported in Newcastle-on-Tyne, England in 1926 (place, from where disease got its name) and on the island of Java, recently part of Indonesia, while some suggestions revealed that there have been some previous outbreaks. It seems that firstly the disease spread quickly in Asia [2]. Current study was planned to assess the current status of ND in Pakistan.

Etiology

The causal agent of this contagious disease is the virulent ND virus (vNDV), which belongs to the order *Mononegavirales*, genus *Avulavirus*, subfamily Paramyxovirinae and family Paramyxoviridae. This virus is single stranded, negative sense, filamentous RNA [3]. On the basis of antigenic variation, avian paramyxoviruses are categorized into different groups as APMV-1, APMV-2, APMV-3, APMV-4, APMV-5, APMV-6, APMV-7, APMV-8, APMV-9, APMV-10, but only APMV-1 is responsible for ND in chickens (Table 1) so, it can be said that vNDV and APMV-1 are the same name of this disease [4].

Virus strain	Natural hosts	Other host (if any)	Remarks
APMV-1 (Newcastle disease virus)	Numerous	-	Varies from extremely pathogenic to in-apparent, depending on strain and host
APMV-2/chicken/California/ Yucaipa/56	Turkeys, passerines	Chickens, psittacines,	Mild respiratory disease
APMV-37turkey/ Wisconsin/68	Turkeys		Mild respiratory disease
APMV-3*/parakeet/ Netherlands/449/75	Psittacines, passerines	-	No infections of poultry reported
APMV-4/duck/Hong Kong/ D3/75	Ducks	Geese	-
APMV-5/budgerigar/Japan/ Kunitachi/74	Budgerigars	Lorikeets	-
APMV-6/duck/Hong Kong/199/77	Ducks	Geese, rails, turkeys	Mild respiratory disease and slightly elevated mortality in turkeys: no disease in ducks or geese
APMV-7/dove/ Tennessee/4/75	Pigeons, doves	Turkeys, ostriches	Mild respiratory disease in turkeys
APMV-8/goose/ Delaware/1053/76	Ducks, geese	-	-
APMV-9/domestic duck/New York/22/78	Ducks	-	-

Table 1: *Serotypes/groups/subtypes of avian paramyxovirus.*

**Serological tests can distinguish among turkey and psittacine isolates [16]*

Transmission

The virus is spread by inhalation or ingestion, which is transmitted according to its pathotype. Based on the disease severity, NDV can be classified into three main pathotypes, mild or unclear respiratory disease is caused by lentogenic strains, moderate respiratory and nervous signs with some mortality are due to mesogenic strains and severe neurological signs, intestinal lesions with high mortality (up to 100%) are caused by the viscerotropic or neurotropic velogenic strains. Velogenic viruses are further classified on basis of predominant clinical signs produced in infected birds, into viscerotropic velogenic (VVND), neurotropic velogenic (NVND) and pneumotropic velogenic (PVND) strains. Direct contact with carrier or diseased birds is the main source of transmission. Carrier birds shed virus in the feces and disease may also be transmitted through nasal secretions, contaminated water, food, equipment's and fecal material [5].

Host range

According to world organization for animal health (WHO), ND is highly contagious disease affecting more than 250 avian species around the globe. Some NDV susceptible host range around the globe is represented in table 2.

Host	Scientific Names	Genotype	Country	Year	Virulence	Accession No.	References
Chicken	*Gallus gallus domesticus*	IX	China	1986	Virulence	FJ436303	[17]
Crested Ibis	*Nipponia nippon*	VIId	China	2013	Virulence	JX855036	[18]
Sparrow	*Passer domesticus*	VII	China	2005–2007	Virulence	FJ938175	[19]
Wild pigeon	*Columba livia*	VI	China	2003	Virulence	HM063425	[20]
Water rail	*Rallus aquaticus*	I	China	2005	Avirulence	HM063424	[20]
Penguin	*Spheniscidae*	VII	China	1999	Virulence	JN599167	[21]
Muscovy duck	*Cairina moschata*	VII	China	2002	Virulence	FJ872531	[21]
White-cheeked starling	*Sturnus cineraceus*	IX	China	2008	Virulence	KC424431	[22]
Spotted dove	*Spilopelia chinensis*	IX	China	2008	Virulence	KC934170	[23]
Blackbird	*Turdus merula*	IX	China	2008	Virulence	KC934169	[23]
Pheasant	*Phasianus colchicus*	VII	Pakistan	2011	Virulence	JX854452	[24]
Peafowl	*Pavo cristatus*	VII	India	2012	Virulence	KJ398400	[25]
Crane	*Gruidae*	-	India	1992	Avirulence	KJ627773	[25]
Guinea fowl	*Numididae*	-	India	2000	Virulence	AY581302	[26]
Japanese quail	*Coturnix japonica*	VII	India	2003	Virulence	KF740478	[27]
Ruddy turnstone	*Arenaria interpres*	Ia	USA	2002	Avirulence	EF564817	[28]
Pelican	*Pelecanus*	-	USA	2008	Virulence	JN941993	[29]
Herring gull	*Larus argentatus*	VII	USA	2010	Virulence	JN255779	[30]
Black-backed gull	*Larus marinus*	VII	USA	2010	Virulence	JN255778	[30]
Double-crested cormorant	*Phalacrocorax auritus*	VII	USA	2010	Virulence	JN255774	[30]
Mottled duck	*Anas fulvigula*	IIa	USA	2001	Avirulence	GQ288391	[31]
Northern pintail	*Anas acuta*	IIa	USA	1987	Avirulence	GQ288378	[31]
Red knot	*Calidris canutus*	Ia	USA	2001	Avirulence	EF564816	[28]
Mallard	*Anas platyrhynchos*	IIa	USA	1999	Avirulence	GQ288389	[31]
Spectacled eider	*Somateria fischeri*	Ib	USA	2007	Avirulence	KC503422	[32]
Slaty-backed gull	*Larus schistisagus*	Class I	USA	2007	Avirulence	KC503482	[32]
Scarlet macaw	*Ara macao*	Vb	Mexico	2009	Virulence	KC808510	[33]
Egret	*Ardea alba*	II	Mexico	2009	Avirulence	KC808497	[33]
Robin	*Turdus grayi*	II	Mexico	2009	Avirulence	KC808491	[33]
Yellow-napped parrot	*Amazona auropalliata*	II	Mexico	2009	Avirulence	KC808490	[33]
Hawk	*Buteo brachyurus*	II	Mexico	2009	Avirulence	KC808489	[33]
Chachalaca	*Ortalis vetula*	Ia	Mexico	2009	Avirulence	KC808494	[33]
Guan	*Penelopina nigra*	Ia	Mexico	2009	Avirulence	KC808498	[33]
Cockatoo	*Cacatuidae*	VIId	Indonesia	1990	Virulence	AY562985	(34]
Finches	*Fringillidae*	Ve	UK	1997	Virulence	AF109886	[35]
Goosander	*Mergus merganser*	Vb	UK	1996	Virulence	AF091623	[35]
Ostrich	*Struthio camelus*	VII	Iran	2011	Virulence	JF820295	[36]
Spur-winged goose	*Plectropterus gambensis*	I	Nigeria	2008	Avirulence	HG326606	[37]
Teal	*Anas crecca*	Class I	France	2010	Avirulence	JQ013039	[38]
Sterna	*S. hirundo*	Vb	Russia	2001	Avirulence	AY865652	[39]

Table 2: *NDV host range around the world.*

Clinical signs

Clinical signs are the first diagnostic tool for the detection of any disease. ND birds showed mild to severe clinical signs figure 1 [6-8].

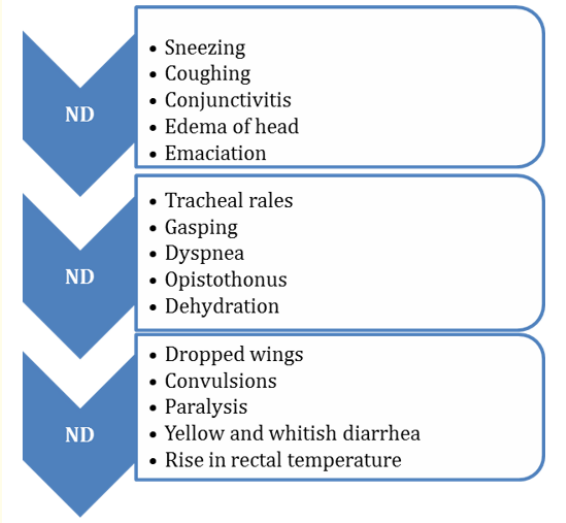

Figure 1: *Clinical Signs in ND infected birds.*

Clinical signs on basis of pathotype

On the basis of pathotypes, disease condition or stage can be diagnosed either it is mild, moderate or severe. Clinical signs related to Velogenic Newcastle disease [9], Mesogenic Newcastle disease [10] and Lentogenic Newcastle disease can be seen in figure 2 [11].

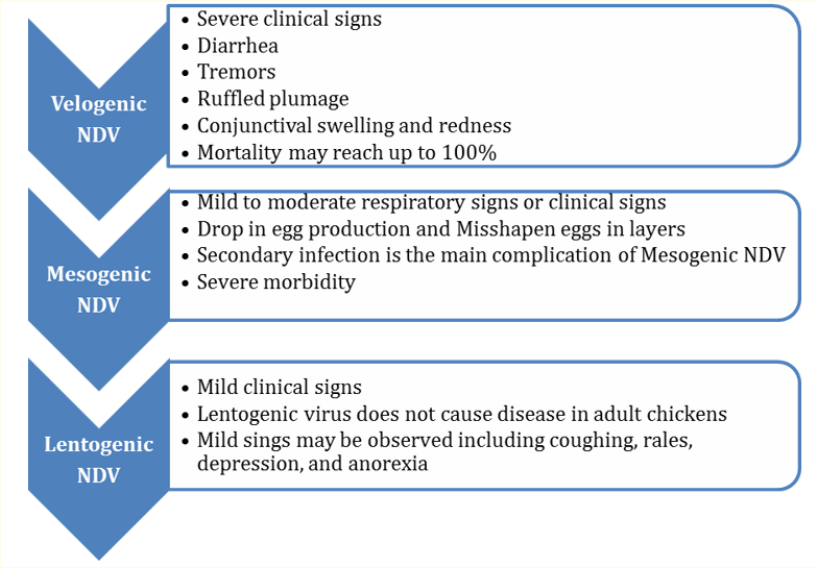

Figure 2: *Clinical signs on the basis of pathotypes.*

Gross and histopathological lesions

Necropsy practice is considered as one of the best tool to observe typical lesions of any disease. Some typical gross lesion related to ND are represented in figure 3 while microscopic/histopathological lesions are described in figure 4 [7,8].

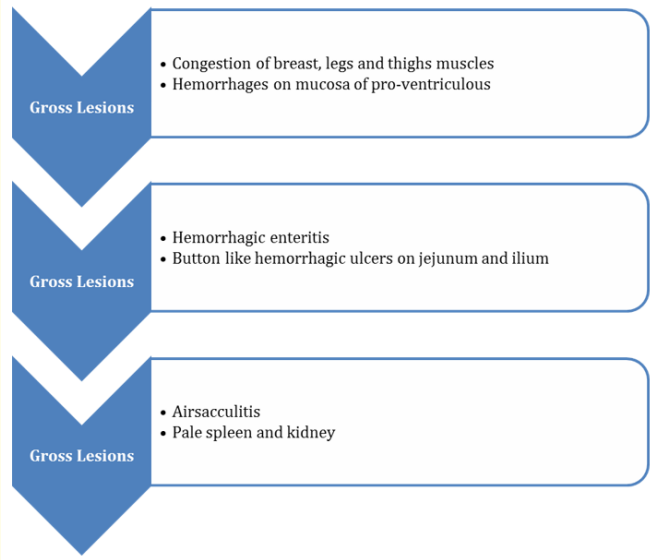

Figure 3: *Gross lesions observed in ND infected birds.*

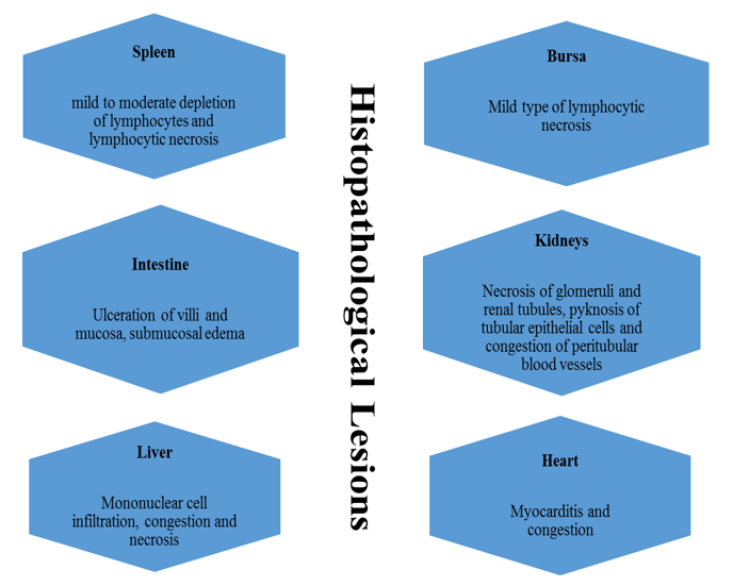

Figure 4: *Histopathological lesions in ND infected birds.*

Diagnostic tools

ND can be diagnosed on basis of clinical signs, symptoms and postmortem lesions. But the clinical signs and gross lesions cannot considered reliable because these may confuse with some other pathogens like ND and Avian influenza. Consequently, tentative diagnosis of ND requires laboratory tests for confirmatory detection of ND. Nasal discharge, fecal droppings, blood or internal organs such as spleen, liver, lungs, trachea and intestinal contents of sick or dead birds can be collected for isolation of ND virus. Virus isolation and identification is regarded as the confirmatory test for ND [12-14]. The confirmation of ND virus can also be done through Polymerase chain reaction (PCR), Haemagglutination inhibition (HI) and Enzyme linked Immunosorbent assay (ELISA) etc. The pathogenicity of ND virus can be checked through intra-cerebral pathogenicity index (ICPI), intra-vertebral pathogenicity index (IVPI) or mean death time (MDT) [15].

ND status in Pakistan

Newcastle disease has been reported throughout the world. However, it causes a great economic loss to poultry sector of Pakistan. The mortality may reach up to 100%. Status of ND in Pakistan can be seen in table 3.

Region/City/State	Bird type	Number of flocks/ farms/ samples	ND status (%)	Reference
Mansehra Khyber - Pakhtunkhwa Pakistan	Broiler birds	248 serum samples (144 vaccinated and 104 non - vaccinated birds)	84% sero - positive in non - vaccinated birds and 97% in vaccinated flocks and level of vaccinated birds was unsatisfactory due to poor strategies	[40]
Khushab District of Pakistan	Broiler and layers	360 poultry farms	7.85% in broilers and 7.92% in layers. This study was dependent on history, clinical and necropsy findings, cultural and biochemical characterization	[41]
Tharparker, Pakistan	Peacocks	1000 peacocks	30% ND in 2012 and 8.6% in 2013. Different status at different age, sex, feeding, treatment, topography, weight, mating, village. Mortality may reach up to 100%.	[42]
Pakistan	Chickens	137 - infected samples and 376 - healthy birds serum samples	19.7% in infected and 3.8% in healthy birds which leads to high morbidity and mortality	[43]
Faisalabad and surrounding districts	Broilers and layers	803 serum samples divided into three age groups (0 - 3, 3 - 5 and 5 - 7 weeks). Level of protection in vaccinated birds was unsatisfactory in broilers as compared to layers	Haemagglutination Inhibition test was performed and Calculated geometric mean titers for broilers groups, 0 - 3, 3 - 5 and 5 - 7 weeks of age were 11.91, 10.01 and 15.85, respectively. While for layer groups, 17 - 27, 27 - 37 and 37 - 47 weeks of age were found to be 134.89, 153.46 and 149.62, respectively. ND can cause high mortality in chickens.	[44]

Table 3: *ND status in Pakistan.*

Preventive measures

To prevent the ND infection, strict measures should be adopted to minimize the economic losses. Therefore, vaccination is the successful approach to prevent the ND infection and to boost the immune system of birds so, properties of live, inactivated and vectored Newcastle disease vaccines can be seen in table 4. Improper vaccination may result in outbreak of ND. Proper management, by reducing stress on

birds, proper disposal of dead birds, routine screening of all birds, by controlling secondary infections, regular monitoring of carrier birds, good hygienic and biosecurity measures are basic strategies to prevent ND [15].

Properties content	Live	Inactivated	Vectored
Storage and constitution	Frozen, freeze - dried; chilled, liquid	Chilled, suspension, emulsion	frozen, cryo - frozen (liquid nitrogen)
Adjuvants	No	Yes	No
Administration route	Mass (spray, aerosol, drinking water) or individual (eye drop, injection)	Injection	In ovo, individual (eye drop, injection subcutaneous or wing - web) or mass (spray, aerosol) depending on the vector
Duration of immunity	Short	Long	Long
Response to the vaccine	systemic and local	Systemic	systemic and local
Antibody immune response	IgY, IgM, IgA	IgY, IgM	IgY, IgM, IgA (depending on the vector and route of administration)
Cell - mediated immune response	Strong	Weak	strong, for Newcastle disease virus (NDV) - vectored
Affected by maternal antibodies	Yes	Yes	Yes
Affected by pre - existing antibodies from previous vaccinations	Yes (depends, if induced by live vaccines)	depending on the level of antibodies	Yes (depends, if induced by live vaccines)
Protection onset	2 - 3 weeks	3 - 4 weeks	4 - 5 weeks
Clinical signs after vaccination	Yes, possible but depends on level of immunity and age	No	-
Thermo-stability	No	No	No
Cost	Less expensive	More expensive	Variable
Vaccine strain	I - 2 V4 PHYLMV42 Ulster, LaSota B1 VG/ GA Clone 30	Any	Any

Table 4: *Characteristics of live, inactivated and vectored ND vaccines.*

Conclusion

Pakistan is a developing country and ND is a major risk to the poultry and poultry farmers with huge economic losses. Currently, there is low production of poultry meat so, high cost of animal protein available as beef and mutton sources in Pakistan. Raising of poultry on latest and modern ways and preventing the disease by adopting preventive measures, can reduce the economic losses of poultry industry. Level of protection of vaccinated and non-vaccinated birds should be checked routinely and boost up if needed. Disease can be minimized by adopting proper management and biosecurity measures. Proper management prevents the transmission of diseases and properly educate the farmer about the biosecurity measures. Controlling of ND can be profitable for poultry farmers and per capita availability of cheap animal protein can be increased as well. Moreover, routine examination of poultry flocks should be done to control the disease at early stages and to minimize the economic losses. However, more epidemiological studies should be reported to check the current disease status in specified regions.

Bibliography

1. Sadiq M. "Pakistan poultry sector still on an upward swing". *World Poultry* 20 (2004): 10-11.
2. Alexander DJ. "Newcastle disease - The Gordon Memorial Lecture". *British Poultry Science* 42.1 (2001): 5-22.
3. Liu HL., *et al*. (2008) "Molecular characterization and phylogenetic analysis of new Newcastle disease virus isolates from the mainland of China". *Research in Veterinary Science* 85.3 (2008): 612-616.
4. Czegle di A., *et al*. "Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications". *Virus Research* 120.1-2 (2006): 36-48.
5. Alexander DJ. "Newcastle disease and other avian Paramyxoviridae infections". In BW Calneck (Ed.), Diseases of Poultry 10th edition, Ames, IA: Iowa State University Press (1997): 541-569.
6. Adene DF., *et al*. "Immunogenicity and safety of new viscerotropic ND vaccines". In proceedings of 13th congress of the world Veterinary poultry association July 19-23 Denver (2003): 143-144
7. Abdu PA., *et al*. "The Epidemiology and clinicopathological manifestation of Newcastle disease in Nigerian local chickens". In: proceedings of 41th congress Nigerian Veterinary medical association 22nd to 26th November 2004 NVRI (2004): 57.
8. Oladele SB., *et al*. "Haemagglutination inhibition antibodies, rectal temperature and total protein of chickens infected with a local Nigerian isolates of velogenic Newcastle disease virus". *Veterinary Research Communications* 29.2 (2005): 171-179.
9. Alexander DJ. "Newcastle disease, other avian paramyxoviruses, and pneumovirus infection". In: Disease of poultry, ed. Shaif YM, Barnes HJ, Glisson JR, *et al*. 12th edition., Blackwell, Oxford, UK (2003): 75-100.
10. Kommers GD., *et al*. "Pathogenesis of chicken-passaged Newcastle disease viruses isolated from chickens and wild and exotic birds". *Avian Diseases* 47.2 (2003): 319-329.
11. Kotani T., *et al*. "Pathological changes of tracheal mucosa in chickens infected with lentogenic Newcastle disease virus". *Avian Diseases* 31.3 (1987): 491-497.
12. Cattoli G., *et al*. "Newcastle disease: a review of field recognition and current methods of laboratory detection". *Journal of Veterinary Diagnostic Investigation* 23.4 (2011): 637-656.
13. Alexander DJ., *et al*. Newcastle disease and other avian paramyxoviruses". In: A laboratory manual for the isolation, identification and characterization of avian pathogens. 5th Edition (2008): 135-141. American Association of Avian Pathologists. Athens, GA
14. Alexander DJ., *et al*. "Avian paramyxovirus type 1 infection of racing pigeons: 3 epizootiological considerations". *Veterinary Record* 115.9 (1984): 213-216.
15. Sharif A., *et al*. "Prevention and Control of Newcastle Disease". *International Journal of Agriculture Innovations and Research* 3.2 (2014): 454-60.
16. Alexander DJ. "Newcastle disease and other avian paramyxoviruses". *Revue Scientifique et Technique Office International des Epizooties* 19.2 (2000): 443-462.
17. Qiu X., *et al*. "Entire genome sequence analysis of genotype IX Newcastle disease viruses reveals their early-genotype phylogenetic position and recent-genotype genome size". *Virology Journal* 8 (2011): 117.

18. Chen S., *et al*. "Phylogenetic and pathogenic analyses of two virulent Newcastle disease viruses isolated from Crested Ibis (Nipponia nippon) in China". *Virus Genes* 46.3 (2013): 447-453.

19. Zhu W., *et al*. "Phylogenetic and pathogenic analysis of Newcastle disease virus isolated from house sparrow (Passer domesticus) living around poultry farm in southern China". *Virus Genes* 40.2 (2010): 231-235.

20. Cai S., *et al*. "Genetic characterization and evolutionary analysis of 4 Newcastle disease virus isolate full genomes from water birds in South China during 2003-2007". *Veterinary Microbiology* 152.1-2 (2011): 46-54.

21. Shi SH., *et al*. "Genomic sequence of an avian paramyxovirus type 1 strain isolated from Muscovy duck (Cairina moschata) in China". *Archives of Virology* 156.3 (2011): 405-412.

22. Duan X., *et al*. "Characterization of genotype IX Newcastle disease virus strains isolated from wild birds in the northern Qinling Mountains, China". *Virus Genes* 48.1 (2014): 48-55.

23. Liu H., *et al*. "Phylogenetic characterization and virulence of two Newcastle disease viruses isolated from wild birds in China". *Infection, Genetics and Evolution* 20 (2013): 215-224.

24. Shabbir MZ., *et al*. "Complete genome sequencing of a velogenic viscerotropic avian paramyxovirus 1 isolated from pheasants (Pucrasia macrolopha) in Lahore, Pakistan". *Journal of Virology* 86.24 (2012): 13828-13829.

25. Desingu PA., *et al*. "A rapid method of accurate detection and differentiation of Newcastle disease virus pathotypes by demonstrating multiple bands in degenerate primer based nested RT-PCR". *Journal of Virological Methods* 212 (2015): 47-52.

26. Kumar S., *et al*. "Pathogenicity of avian paramyxovirus serotype-3 in Chickens and Turkeys". In: Perez- Marin CC (edition), A Bird's-Eye View of Veterinary Medicine, In Tech, Croatia (2012): 587-596.

27. Bhuvaneswari S., *et al*. "Complete genome sequence of a Newcastle disease virus from a Coturnix coturnix japonica (Japanese Quail) covey in India". *Genome Announcements* 2.3 (2014): e00374-e00314.

28. Dhama K., *et al*. "Pathogens transmitted by migratory birds: Threat perceptions to poultry health and production". *International Journal of Poultry Science* 7.6 (2008): 516-525.

29. Hines NL., *et al*. "An rRT-PCR assay to detect the matrix gene of a broad range of avian paramyxovirus serotype-1 strains". *Avian Diseases* 56.2 (2012): 387-395.

30. Diel DG., *et al*. "Genetic diversity of avian paramyxovirus type 1: Proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes". *Infection, Genetics and Evolution* 12.8 (2012): 1770-1779.

31. Zhang GZ., *et al*. "Serological survey on prevalence of antibodies to avian paramyxovirus serotype 2 in China". *Avian Diseases* 51.1 (2007): 137-139.

32. Ramey AM., *et al*. "Genetic diversity and mutation of avian paramyxovirus serotype 1 (Newcastle disease virus) in wild birds and evidence for intercontinental spread". *Archives of Virology* 158.12 (2013): 2495-2503.

33. Cardenas Garcia S., *et al*. "Molecular epidemiology of Newcastle disease in Mexico and the potential spillover of viruses from poultry into wild bird species". *Applied and Environmental Microbiology* 79.16 (2013): 4985-4992.

34. Wise MG., *et al*. "Development of a real-time reverse-transcription PCR for detection of new castle disease virus RNA in clinical samples". *Journal of Clinical Microbiology* 42.1 (2004): 329-338.

35. Alexander DJ. "Newcastle disease in the European Union 2000 to 2009". *Avian Pathology* 40.6 (2011): 547-558.

36. Ghalyanchi-Langeroudi A., *et al*. "Sequence Analysis of Fusion Gene of Newcastle Disease Viruses Isolated from Ostrich (Struthio camelus) in Iran". *Indian Journal of Virology* 5.3 (2011): 12-17.

37. Snoeck CJ., *et al*. "Characterization of new castle disease viruses in wild and domestic birds in Luxembourg from 2006 to 2008". *Applied and Environmental Microbiology* 79.2 (2013): 639-645.

38. Briand FX., *et al*. "Complete genome sequence of a novel avian paramyxovirus". *Journal of Virology* 86.14 (2012): 7710.

39. Usachev EV., *et al*. "Molecular genetic characteristics of the Newcastle Sterna/Astrakhan/Z275/2001 virus isolated in Russia". *Molekuliarnaia genetika, mikrobiologiia i virusologiia* 1.1 (2006): 14-20.

40. Khurshid S., *et al*. "Serological Status of Newcastle Disease Virus in Live Broiler Birds of Mansehra Khyberpakhtunkhwa Pakistan". *Advances in Biotechnology and Microbiology* 11.2 (2018): 555810.

41. Abbas G., *et al*. "Incidence of poultry diseases in different seasons in Khushab district, Pakistan". *Journal of Advance Veterinary and Animal Research* 2.2 (2015): 141-145.

42. Mustafa I., *et al*. "Newcastle disease as an emerging disease in peacocks of Tharparker, Pakistan". *The Journal of Infection in Developing Countries* 9.8 (2015): 914-916.

43. Alam J., *et al*. "Dot-ELISA for Newcastle Disease, Infectious Bursal Disease and Mycoplasmosis". *Pakistan Journal of Zoology* 44.5 (2012): 1301-1305.

44. Numan M., *et al*. "Serologic status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts". *Pakistan Veterinary Journal* 25.2 (2005): 55-58.

Volume 4 Issue 9 November 2019