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Prevalence of Newcastle Disease Virus in Pakistan, its present status and future challenges

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Abstract

Newcastle disease is caused by Newcastle Disease Virus (NDV) leads to severe morbidity and mortality in poultry throughout the world and considered as lentogenic, mesogenic or velogenic based on the mean death of the chicken embryo. The NDV velogenic strain is deadly endemic in Pakistan. Poultry is considered as the second major industry in Pakistan having annual growth of 8-10%. Unfortunately, the increase of NDV cases leads to severe cost impact, loss of production and livelihood. This review highlights the current status and epidemiology of NDV in Pakistan. Various genotypes and sub-genotypes have been identified in Pakistan. Various ND cases have been reported in Pakistan which has very bad consequences on the economy and dealing of poultry products.



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Introduction

Newcastle is a highly contagious disease results in severe economic problems for poultry industry. The causative agent for Newcastle disease (ND) was first observed in Newcastle, England [1], and named as Newcastle disease. Next year, the same disease was reported in India Ranikhet and therefore named as Ranikhet in India [2]. It has also been reported that similar outbreak has occurred in 1926 and in 1898, Isles of Scotland and was responsible for the major death of fowl. In 1948, major cases of ND have been identified in Egypt [3]. According to World Organization for Animal Health (OIE), the ND is the third most devastating poultry disease and reported in 109 countries [4]. The velogenic strain of ND has transmitted more between the chicken farms [5]. This virus has been spread throughout the continents of the world except for Antarctica. It causes destructive economic effects on global domestic poultry production [6] as it infects both wild and confined birds. Nearly four panzootic have been identified due to ND since 1927 [7]. It is an alarming condition that ND has the ability to to infect the vaccinated chicken depends on improper vaccination or mutations [8]. ND is highly similar to the Avian paramyxovirus type I however now it is referred to as avian avulavirus [9] due to differences in taxonomy [10]. ND can be differentiated into lentogenic, mesogenic, and velogenic based on its pathogenicity [11].

Genetics of virus and pathogenicity

This avian avulavirus is a negative sense, having 15200 nucleotides of single-stranded RNA and can infect more than 241 species of the birds [12]. ND can infect a wide range of birds due to high pathogenicity and genetic diversity [11, 13, 14]. The cattle egrets are the source of transmission of velogenic strain in other birds as susceptible to velogenic ND [15]. The domestic poultry of six continents including Africa, America, Asia, Australia, and Europe have been affected. Newcastle Disease Virus (NDV) is composed of 6 genes, structural and other proteins [16] such as nucleocapsid protein (NP) having a structural and non-structural role in infection, phosphoprotein (P), matrix protein (M) having role of viral assembly, fusion protein, hemagglutinin-neuraminidase protein (HN), and an RNA polymerase (L) having the role of transcription of mRNA of NDV [17, 18]. Host cell

protease split precursor fusion protein, F0 into F1 and F2 proteins lead to begin the infection with the fusion of cells and activation of hemolytic properties. At the cleavage site of F protein, the amino acid motif is the site of recognition for protease. The hemagglutinin-neuraminidase promotes fusion activity for virulence of NDV [19]. It plays a significant role by promoting the host cell penetration and preventing the generation of virus from self-agglutination by removing the sialic acid [20, 21]. The penetration mechanism of enveloped viruses includes direct fusion in which the viruses in process of enveloping mingle with the host plasma membrane leads to endocytosis. In the endocytosis mechanism, viruses bind at the host receptor cells result nucleocapsid entrance into the host cell cytoplasm [1, 22]. According to a recent system of classification, there are two classes of NDV isolates. Class I has a single genotype and class II has eighteen genotypes [23]. Various genotypes and subgenotypes such as VI, VIIId, VIIIf, VII, XIIIb have been reported, including highly pathogenic subgenotype VIIIi AAvV1s in Pakistan from peacocks, pheasants, parrots, and pigeons. Europe is highly affected by genotype VII. In Iran and India, the subgenotype VIIb has prevailed.

Epidemiology (Pakistan)

In 1963, ND was detected in Pakistan, and since then it is constantly infecting the commercial and layer flocks. The major target of NDV is poultry flocks, while it also infects wild birds, including sparrows, hawks, waterfowls, and pigeons [24, 25]. The movement of these wild birds can transfer the NDV into a new host and to other geographical area [26].

1986 to 2008

The most common source of its spread is the movement of the birds and in trade practice transport of infected birds [11, 27]. The NDV in 9-10 day old chicken embryonated eggs causes hemagglutination [28]. A study is conducted in the Sheikhpura district to check the susceptibility in local and Fayoumi breeds [29]. The result showed that the 57% of Fayoumi was more susceptible and Desi breeds were at 43%. Various infectious agents such as NDV, AI, *E.coli*, and salmonella are also involved in respiratory outbreaks in chickens. Poor management, poor vaccination, and poor feeding practices, as well as migration of birds, are the basic factors for the prevalence of ND [30].

From June 2007 to May 2008 in the livestock department of Khushab district, a laboratory diagnosis of poultry disease was carried out to study the poultry diseases in broilers and layers. The result showed most affected with ND was broilers and the average rate was 7.85%. ND virus affected both layers and broilers. Thus it is mandatory to improve vaccination, management, and biosecurity practices [31]. From 50 samples, 40% of chickens were positive and 4% were immune from NDV. From 2007 to 2008 the southern region of Pakistan is mostly affected by ND [32].

2008 to 2021

From 2008 to 2010, ND was top-ranked in rural poultry of Pakistan as seroprevalence was noted 40.5% in district Faisalabad [33, 34]. It was found that 41.33% of unvaccinated chickens were containing antibodies against NDV. The unvaccinated chickens were more susceptible to ND [35]. During this tenure, the prevalence of ND was 100% in severe forms. In 2008 a study from 457 families who were producing rural birds showed that ND affected 65.8% of poultry birds and the mortality outbreaks were found 54%. A commercial hub for poultry production, Rawalpindi was suddenly affected by outbreaks of NDV during April 2010. The World health organization for animal health has reported various cases of ND in Pakistan and neighboring countries from 2009 to 2012. The most affected areas were India and Iran and both the countries are neighbors of Pakistan. In contrast, it is believed novel NDV (5i) emerged from Pakistan [36]. In Jallo Wildlife Park, Lahore, the serotype AAVV1 severely infected the wildlife during 2012. 190 peacocks died due to it with a 100% mortality rate within one week. This serotype is isolated and identified through a serological test such as ELISA, real-time PCR, and HI test [37]. In this tenure, approximately 167 peacocks also died due to NDV in seven southern districts of the Sindh province [36]. From the sudden five outbreaks of NDV during 2010 to 2011, there were 130 samples from the trachea, lung, spleen, cloaca, and blood were collected. The results showed a 10 to 15% mortality rate per day in broiler and 5-8% per day in layer flocks. All the infected birds showed symptoms of ND including dullness, depression, and difficulty in respiration. This study showed that the genotype VII is still prevalent in poultry of Asia, and also identified that the genotype VII is not evolutionary different from the isolate of 1990s. Various reports showed that the

NDV was prevalent during 2010-2012 in both vaccinated and unvaccinated birds in Punjab. So for screening 113 samples having NDV of birds including pigeons, turkeys, and peacocks from different districts of Punjab, Khyber Pakhtunkhwa, Sindh, Baluchistan have been tested. Real-time PCR of samples showed 23 samples positive in both F and M genes. The value of the mostly positive sample showed between 25 and 30.

The subgenotype of VIIIi, Avian Avulavirus 1 (AAvV-1) was constantly infecting the commercial and poultry chickens (2011-2016). This showed the relation between ND in poultry and infections with virulent AAvV-1 strains in other avian species. This study showed the endemicity of ND due to the number of hosts and optimum environments [38].

Transmission (Pakistan)

NDV transmissions by inhalation or ingestion and respiratory secretions from infected birds have been reported [39]. NDV can survive many months on chicken skin or bone marrow if the optimum temperature is provided. Its survival mechanism is different according to season such as 7 days in summer, 14 days during spring, and 30 days in winter [11]. The survival pattern of NDV varies, with different birds as it survives 1-2 weeks in gallinaceous birds, and 1 year in psittacine birds. Mostly NDV transmits through contaminated feed, water, feeders, utensils, and eggs crates. The clinical symptoms showed differ in vaccinated and unvaccinated birds. The mutating ability of NDV increases its host range and transmission.

Partridges and Pheasants

Partridges and Pheasants have the same signs as chicken, and they are more susceptible to NDV. It results in the lesion, severe signs, and sudden death. In 2012 almost 67 pheasants died due to an AAV-1 outbreak in a wildlife farm in Lahore. The fatality rate including loss of 60% of susceptible birds was 80% [40].

Ducks, Waterfowl, and Peacocks

NDV can infect ducks, and the mesogenic strain shows neurological symptoms in ducks. Generally, no symptoms appeared when ducks were infected with NDV.

Waterfowl birds during the tenure of the winter season enter Pakistan and migrate to the whole country. These migratory birds also carry avian viruses and spread to the other birds. These birds enter in Pakistan from different destinations such as the Hindu Kush, Karakorum, and Suleiman ranges and they use Indus Flyway [38]. NDV genotype VII and subgenotypes 'e' infect the peacocks. The NDV has been spread throughout the country and it infects the birds. In Hyderabad June 2013, it is reported that five dozen peacocks have been died due to NDV. Deadly ND affects the nervous system of those having a deficiency of vitamins and minerals.

Vaccines

Vaccines are the only weapon that can control infectious diseases of poultry. There is a need to minimize the vulnerability level of these diseases by applying a proper vaccination scheme in domestic poultry. For healthy chickens, it is a major source of infection if ND spreads in other avian species such as pigeons, sparrows and crows. A low costly Lasota strain vaccine is the most popular to prevent the NDV pandemic. For the prevention of this disease in chickens, live vaccines and killed oil-based vaccines are being used in Pakistan and also in other countries [41]. Despite the use of the vaccine, an outbreak is also occurring in different countries results in huge economic losses. To diagnose this disease, there is a requirement of history, clinical signs of this disease, and laboratory approval authentication [42]. To minimize ND viral multiplication and inactivated ND vaccine with a proper vaccination schedule is considered to be more effective.

Are vaccines effective or not?

NDV virulent and its contact with poultry can be controlled by proper administration and medical biosafety. Since the 1650s, live and inactivated vaccines have been used. Infection of NDV causes death due to inappropriate vaccination. The presences of antibodies in birds neutralize the effect of ND and reduce its efficacy. Poorly vaccinated birds have been affected by neurological, reproductive, and respiratory system-associated diseases [43]. There are three methods to control ND by using vaccines, put an end to the malady, minimize multiplication of virulent strain and increase the amount of dose against the challenged virus [44]. There is no implementation on the second and third goals due to a lack of tools that measure the

efficacy of vaccines, so only the first target has been investigated for implementation [45]. Due to immunosuppression and other genetic variations in the circulating ND virus, the Lasota strain is not protecting broiler birds.

Economic Impact

The poultry industry is considered as a backbone in commercial progress for agricultural countries like Pakistan. In the period of protein inadequacy, meat and eggs were the major sources of proteins. Poultry production is increasing day by day as every rural and urban family is associated with and meat production is 26.8 percent out of total meat production in Pakistan. According to the economic survey of Pakistan (2011 to 2012) grant of the poultry sector in agriculture was 6.40 percent and in livestock is 11.50 percent, moreover, it had rapid growth of about 8 to 10 percent every year which became a source of employment for 1.5 million people. ND and avian influenza are transmissible and viral diseases that spread rapidly and effects socio-economic progress and poultry products [46].

Diagnostics

Serially used methods and other diagnostic tools have been used to identify the pathogen. Differentiation can be done by inoculating samples of sick and dead birds into embryonated eggs. To determine hours, require for the killing of chicken embryo and to inoculate samples into embryonated eggs and death time is frequently used for the diagnostic methods. In old days chicks, intracerebral pathogenicity index and intravenous pathogenicity index are used to determine prioritized features of clinical signs. Test of choice is done with ICPI and for common tests, IVPI is used. For NDV diagnosis, a diagnostic test is done called hemagglutination assay in which infected flocks are suspended in a solution contains antibiotics to kill the virus. Through estimation of amino acid residues at the fusion site, the live virus is isolated for further pathotyping to determine the virulence strain. To check out NDV-specific antibodies in the host, hemagglutination assay and enzyme-linked immunosorbent assay are used. Due to unchecked vaccination methods, serological diagnostic methods are not used to identify NDV as they do not allow differentiation of antibodies and do not show much efficacy in identification. By using ELISA, many operations monitored the serology of a flock, and

antibody titer for NDV is determined while if it exceeds the normal value then further estimation is done. Abnormality can be identified rapidly by the use of this monitoring method [47].

Prevention and control

To prevent the outbreak of NDV, vaccines are being used. Birds and chicks are vaccinated against ND. This vaccination only protects birds and chicks however does not control the replication of the virus [48]. The new strain of ND virus is the main reason for vaccine failure and desi birds are susceptible to this strain. There are many routes from which vaccination can be done. It can be done in the form of eye drops *via* spray and through drinking water. If vaccination is done in the form of drinking water, then before 24 hours there is a need to stop the addition of drugs and other antiseptics in drinking water and after vaccination, this addition can be restarted. There is a chance of outbreak occurrence in flocks even after vaccination due to inappropriate methods of vaccination [49]. Second-rated vaccines, poor weather conditions, use of vaccines after expiry dates, transferring of birds after vaccination, and poor stocking facilities are the major cause of vaccine failure. To get from spreading of ND, there is a need for vaccine in monitoring quality and dead birds must be buried in a ditch that can be constructed in boundary walls of farm areas [50].

Commercial poultry

Regardless of vaccination strategy accomplishment, ND disease seems to appear in commercial broilers, and it is the major transmissible outburst in commercial poultry flocks in Pakistan leads to economic loss. Punjab, Pakistan is considered as a center of poultry because of favorable environmental conditions [36]. Only in Punjab, there is a huge economic loss of 65 million US \$ and 45 million chickens died from September 2011 to Jan 2012. In the pandemic of ND, the role of wild birds is not fully understood, but it is supposed that in the spreading of virus from affected to non-affected farms wild birds play a major role [51]. Early growth in commercial poultry farming started in Pakistan was the result of advanced strategies [52]. Commercial poultry is considered to be an important part of the food processing industry [53]. Pakistan put up with high fodder prices because it remains

unsuccessful in the production of specific-pathogen-free chickens [54].

Risk analysis

There are chances of increasing avian virulent avulavirus strain through seasonal wild avian species of NDV, *via* transferring of live birds, poisonous poultry products, and poor water supplies. Close housing, indiscriminate vaccination, wild birds movement into farms, and insufficient diagnostic facilities are the main causes of NDV in Pakistan. Pakistan needs a comprehensive national policy for a disease-free poultry sector [55].

Domestic backyard poultry management risk

In the period of protein inadequacy, meat and poultry eggs were the major source of protein. It is considered as the backbone of commercial rural poultry and the economy. It plays a major role to enhance the GDP. Rural poultry plays a major role in improving the village economy as it contributes upto 3611 million eggs and 100.41 metric tons of total poultry meat. Poultry production is the major source of income and ND outbreak has insecurity impacts in these small areas. Faisalabad serology of ND in unvaccinated rural poultry is measured as 40.5 percent. According to a report, 41.33 percent of unvaccinated rural chickens had been found to have ND against antibodies [33]. The acute form of ND is the cause of a 100 percent death rate in unvaccinated birds [56].

Disease awareness

There is a lack of knowledge about poultry management in almost 12 percent of families and 88 percent of families have inadequate or low awareness. In 92 percent of families, the administration of birds is the duty of women, and 8 percent of men are responsible for this. The death rate is more in birds that do not have a vaccination against ND as compared to those birds that have ND vaccination [57]. Those farmers that keep their birds in darkened and overcrowded houses have a higher death rate as compared to those farmers that keep their birds in a suitable place of habitation. Improper residence for birds, lack of vaccination, poor weather conditions, lacking feed, overcrowded houses are the main reasons that lead to the higher death rate in birds and

the occurrence of a pandemic [33]. According to a report, there are more chances pandemic when walking birds mix into an already existing group of birds as compared to those hens that want to hatch their eggs in houses [58]. It has been reported that unfavorable poor weather conditions and heat stress become the reasons for lower immune status of birds and cause of outbreak [33, 59].

Gender difference

There is a higher death rate in young birds due to ND as compared to old birds [60, 61]. It is suggested that there is the highest risk of infection among birds of age 16 to 24 weeks. According to Kutubuddin in 1973, when an investigation has been done at the farm of Bangladesh Agriculture University, the female birds have a low effect of NDV as compared to the male birds. According to a recent study, the percentage of death rate is higher among chicks as compared to males and female birds [33].

Laboratory risk

There is a more need to determine the level of biosecurity and biosafety to identify the ND virus which has chances to spread from laboratories. Countries which have lack biosecurity facilities and regional laboratories should send their specimen to the OIE reference laboratory. ND is the major cause of economic loss and animal diseases. There is a need to spread awareness through efficient laboratory methods to lower the level of this disease. To confirm field doubt, to identify the virus, and to satisfy international laboratory needs, laboratory testing is necessary. To gather the whole picture and to identify threats, there is a need to use pathological observations with virology and molecular biology laboratory methods. For NDV diagnosis, genetic variations show the main constraints of advanced molecular techniques.

Biosecurity

Proper biological safety and control of migration of birds that has NDV infection are necessary to prevent ND. Improper biological security is the main reason for the spreading of ND outbreak. There is a need to keep more distance between newly constructed poultry farms and the old ones. For prevention, a proper biological safety system is necessary to control the spreading of viral and bacterial diseases.

The movement of wild birds, animals, and common people in farms should not be allowed to control the NDV [50]. There is a need to take strict action against the movement of poultry attendants and electricians in wild birds' poultry without wearing clothes. To work in poultry houses, there is a need to wear clean dresses. Preventive measures should be taken to prevent from spreading of NDV in poultry flocks [62]. For the collection of birds that died from NDV infection, Standard operating procedures should be followed. To get rid of NDV, there is a need for the establishment of sterilization and sanitization and minimizing the number of movements of infected birds.

Proper management

Poor incubation methods, lack of precautionary measures, and poor nurturing of poultry flocks can lead to the occurrence of ND. Nurturing different poultry flocks of different ages can spread the ND disease in birds [62-64]. There is a need to take up the proper administration for outside and inside nurturing of flocks. Complete sanitization of farms during the period of housing poultry groups can be achieved by efficient administration and farming of the same age of bird groups. Before entering farmhouses, trucks that carry fodder bags should be appropriately sanitized and tires should be properly sterilized. Before nurturing new flocks in the sheds, poultry sheds should be properly sanitized. Poor drinking conditions may be the source of infection for birds. In poultry flocks, the major source of infection is poor housing [62].

Future challenges

For developmental ancestry among many pandemics, the virus of Newcastle disease has not been studied periodically. There is a main concentration on the investigation of immunological studies of the virus. There are chances of genetic modifications of the pathogenic strain of this virus because of the overuse of vaccines. It is obligatory that international research should be done on these issues, such as the research on the deadly strain of Newcastle disease virus which can replicate fastly and produce virulent disease, how we can separate NDV from the host and identify it by molecular tools, how DNA testing should improve and arrangement for the study of developmental ancestry, control and transmission of disease and to identify immunogen and host-immune

response strategies, Use of PCR application to measure prevention and control of disease in the future [65].

Conclusion

Avian avulavirus is the cause of ND spreads rapidly and causes the death of poultry species. In the international Office of epizootics, it is obligatory permissible to identify virulent NDV that results in the acute, long term and become a hurdle for international trade. Pakistan needs a comprehensive national policy for a disease-free poultry sector. In developing countries like Pakistan, it is a serious risk for the poultry industry. ND pandemic has been rapidly spread all over Pakistan. There is a need for new strategies to generate vaccines as these old strategies are not effective due to poor conditions. There is a need for new perspectives and the production of safe and strong subordinates to enhance the efficacy of vaccines. There are more benefits of the future ND vaccine as compared to the present vaccine system allows rapid action against new emerging ND strains. It remains a major problem as it is a destructive disease in poultry industries in many countries.

Conflict of interest

The authors declare no conflict of interest.

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