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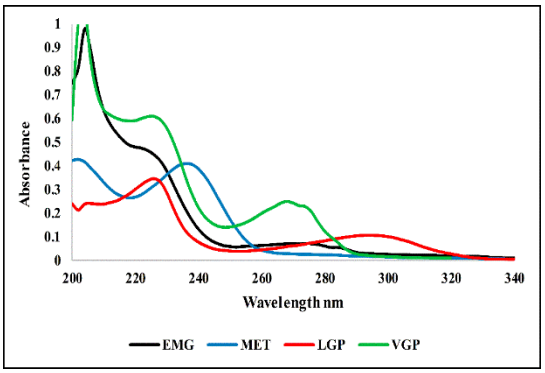

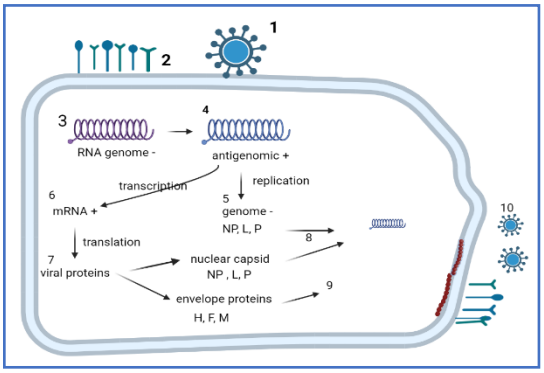

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Newcastle disease: A review

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Abstract

Newcastle disease (ND) is a contagious disease caused by infection with the Newcastle disease virus (NDV), a member of the genus Orthoavulavirus (family Paramyxoviridae). Chickens are the primary infected species, although most domestic and wild birds are susceptible. The disease is endemic in Asia, the Middle East, Africa, and Central and South America, causing significant economic losses in the infected farms through reduced egg production, reduced weight gain, and mortalities. The NDV genome is a negative-sense, single-stranded RNA genome of about 15,190 nucleotides and encodes six structural and two nonstructural proteins. Velogenic NDVs are highly virulent and may cause up to 90% mortalities, while mesogenic strains cause depression, weight loss, reduced egg production, and mortalities around 10%. Infection mainly occurs through inhaling or consuming virus aerosols or contaminated dust. ND is endemic in many parts of Iraq and poses a significant threat to the poultry industry. Immunization is the most common practice to protect chickens in endemic areas. Some compounds were reported to have antiviral activities against the virus in embryonated chicken eggs; however, there is no approved treatment for ND. This review discusses the etiology, epidemiology, replication cycle, pathogenicity, economic impact, and diagnosis of ND, as well as the prevention and control of the disease in poultry.

Introduction

Poultry farming is a significant section of the livestock industry that supplies cheap animal protein to consumers worldwide. The primary threat to the sector, which causes a substantial decrease in production and financial losses, is infectious diseases, particularly viral diseases. Newcastle disease (ND) is a viral illness affecting various bird species, domestic and wild **[1]**. It is one of poultry's most prevalent infectious illnesses and is spread worldwide **[2]**.

The Newcastle disease virus (NDV) belongs to the Paramyxoviridae family and subfamily Avulavirinae and is the cause of Newcastle disease. It was formally renamed avian avulavirus 1 by the International Committee

on Taxonomy of Viruses (ICTV) in 2016 and again as avian orthoavulavirus 1 in 2018 **[3]**. Virions are enveloped pleomorphic particles about 100 to 500 nm in diameter and have about 15.2 kb genome **[4]**.

A disease with enormous economic impact, ND presents a severe danger to poultry management **[5]**. It occurs in many developing countries, primarily in areas where the poultry industry thrives **[6]**. The annual losses from this illness are estimated to be millions of US$ **[7]**. Many NDV strains cause different death rates and sickness in a flock **[8]**, which also causes a decline in egg production **[9]**. The meat quality in most developing countries may be affected by ND infection **[10]**.

ND was first reported in Newcastle-upon-Tyne, England, and the first descriptions were in 1926 **[11]**. However, the disease remains an epizootic in Asia, Africa, and Central and South America, and intermittent epizootics occur in Europe **[12, 13]**. Birds such as pigeons, cormorants, and imported psittacines are the most frequent carriers of virulent NDV, and they have also infected livestock with virulent NDV. In addition, poultry and wild birds, particularly ducks, frequently have low virulent NDV (loNDV) strains, which reduce productivity **[14]**. Humans can be infected with the virus, as conjunctivitis may occur following exposure to high concentrations **[15]**. Conjunctivitis often clears up quickly, although the virus can persist in the ocular discharges for up to one week. In some instances, a mild, self-limiting illness similar to the flu in humans has also been observed **[16]**. No proof supports human-to-human infection, but human-to-bird transfer is possible **[17]**.

ND remains a significant problem encountered in the poultry industry in Iraq and neighboring countries. There is no treatment for the disease, and vaccination is the only way to protect poultry. This review summarizes the etiology, epidemiology, and economic impact of the ND. It also discusses the virus's replication cycle, pathogenicity, diagnosis, prevention, and disease control.

Newcastle Disease's Etiology

ND, a contagious viral illness that affects many bird species, is a significant poultry disease worldwide. Mild to severe cases of the sickness are possible, and exotic ND is a contagious fatal illness that causes many birds to die abruptly without symptoms **[18]**.

The viruses that cause ND belong to the order Mononegavirales, family Paramyxoviridae, subfamily Paramyxovirinae, and genus Orthoavulavirus, with another eight avian paramyxovirus (APMV) serotypes (APMV-2 to APMV-9) **[19]**. The RNA genomes of viruses of the genus Avulavirus are single-stranded, negative sense, and non-segmented **[20]**. The genus Orthoavulavirus contains all APMVs. The virions are filamentous, approximately spherical, and have a diameter of 150 nm or more **[21]**. The approximately 15.2 kb-sized genome harbors the codes of six structural and two nonstructural proteins **[22]**. These proteins are Nucleoprotein (NP), Large RNA polymerase (L), Fusion (F), Hemagglutinin-Neuraminidase (HN), Matrix (M), and Phosphoprotein (P), which run in the 3' to 5' direction **[23]**. NP is determined to be highly immunogenic because it causes chickens to produce antibodies **[24]**. In the P gene, guanines are introduced at the editing site when the mRNA is transcripted to make the proteins W and V **[25]**. The V protein binds zinc and includes a significant amount of cysteine **[3]** and a carboxy terminus with anti-interferon (anti-IFN) activity. The section lets the virus reduce the host's innate immune response **[26]**. NDV also binds the host's

actin protein, essential for viral entrance, replication, and movement across the cell **[27]**. NP is the most common protein in the virus, which gives the helical nucleocapsid shape and is the primary viral genome replication regulator **[28]**. The RNP complex acts as a template for RNA during the latter's synthesis, and it comprises the proteins NP, P, and L, as well as the genomic RNA **[29]**.

NDV strains can be grouped according to their pathogenicity into four types: viscerotropic velogenic, neurotropic velogenic, mesogenic, and lentogenic **[30]**. The strains are also classified into different genotypes according to the F gene's phylogenetic analysis from genotype I to X. Virulent viruses mostly fall in genotypes III to X, while genotypes I and II are used as vaccines **[31]**.

Species Susceptible to ND

Several domestic and wild bird species are susceptible to the virus **[32]**. Turkeys rarely show severe symptoms of illness, and chickens are more susceptible to it **[18]**. Waterfowl and wild birds (order Anseriformes) may have a subclinical virus infection **[33]**. Young cormorants (Phalacrocorax spp.) have shown symptoms of an APMV-1-related illness **[34]**. Pigeons are reportedly vulnerable, and ostriches (order Struthioniformes) have been reported to have the disease. Raptors are typically resistant to ND, but cases of acute sickness were reported in wild ospreys (Pandion haliaetus), white-tailed sea eagles (Haliaeetus albicilla), bearded vultures (Gypaetus barbatus), and several falcon species **[18]**. Pelicans (order Pelecaniformes), owls (order Strigiformes), and gulls (order Charadriiformes) are some other birds that have been linked to NDV **[33]**. The vulnerability of passerine birds (order Passeriformes) varies; some species exhibit no symptoms but still excrete NDV, whereas others may experience severe sickness. Crows and ravens (genus Corvus) have been reported to die from infection. Penguins (order Sphenisciformes) have been known to develop acute ND. Different species and strains of NDV cause different morbidity and mortality rates **[35]**.

Epidemiology

Middle Eastern, African, and Asian poultry growers struggle with ND. In some areas where vNDV is endemic, mesogenic NDV strains are still used as live vaccines. These strains are characterized as virulent due to their high intracerebral pathogenicity index (ICPI) values, which further complicates the problem **[36, 37]**. Evidence shows that the vNDV has spread to countries throughout Asia, Africa, the Middle East, and Europe **[38]**. Numerous major ND pandemics have been documented all over the globe. Beginning in Java, Indonesia, and Newcastle-upon-Tyne, England, the first pandemic occurred in 1926 **[39]** and persisted till the late 1950s **[40]**. The second pandemic occurred in the Middle East in the late 1960s and was transmitted to other nations until 1973. Moreover, the third severe panzootic caused by a neurotropic variant of NDV first appeared in the Middle East around the end of the 1970s, and the disease spread to Europe in 1981, followed by a swift global spread **[41]**. The Far East, South Africa, and Europe experienced the most recent fourth pandemic in the late 1980s **[40]**.

Economic Impact

Poultry diseases negatively influence human well-being, particularly in the countryside, where backyard or village chickens are a significant source of revenue and nutrition **[42]**. Countries with industrialized chicken production spend much money eradicating ND (Table 1) after an outbreak, maintaining ND-free status, and

preventing losses **[43]**. As a result, the poultry sector experiences significant yearly financial losses worldwide **[8]**.

Table 1. The economic impact of ND in different countries

Country	Species	Economic impact	Ref.
Iraq	Broilers	Outbreaks of ND occur throughout the country, including Baghdad, Diyala, Sulaymaniyah, Basrah and Al-Najaf.	***[44-48]***
Pakistan	Broilers	ND is still common and significantly impacts villages where people's income primarily depends on raising chickens. Infections caused a 90% decrease in egg production and the death of 45 million chickens between 2011 and 2012	***[49-51]***
Pakistan	Peacocks	One hundred ninety peacocks perished after the NDV epidemic in Lahore's Jallo Wild Life Park.	***[50]***
Bangladesh	Village chicken	There are both direct and indirect losses. Direct losses are due to expensive treatment and high mortality. Indirect losses are due to the time invested in chicken rearing and production loss, such as decreased egg yield.	***[52]***
Germany, Belgium, Sweden, and Slovenia	Backyard and commercial poultry	Infections resulted in limited marketing opportunities. In addition, backyard poultry vaccination every three months and the flocks' regular supervision are money and time-consuming.	***[13, 53]***
Turkey	Poultry flock	dangers from epidemiology to the poultry sector.	***[54, 55]***
Iran	Broilers	$US 3.86 million in financial costs and losses. With 2.27, 1.11, 0.33, and 0.036 $US million, veterinarian services, medications, vaccination, and cost for 1-day-old chicks were the second highest in 2016-2017.	***[56]***
Oman	Commercial birds	The frequency of the virus and the disease's propagation to commercial flocks are affected by backyard poultry's high seroprevalence of NDV.	***[57]***
Nigeria	Rural birds	Several NDV outbreaks have resulted in substantial mortality rates for local birds.	***[58]***
Egypt	Broiler chicken flocks	Even though Egypt has implemented intensive vaccination programs, NDV outbreaks are still commonly reported, with significant flock losses. In addition, the sporadic use of potent vaccinations, frequent mutations, and the formation of new NDV pathotypes could cause numerous NDV outbreaks.	***[59]***
Kenya	Chickens	NDV is regarded by many as the biggest obstacle to effective smallholder poultry productivity in Africa.	***[60]***
Tanzania	Chickens	It produces a high rate of chicken mortality and is widespread throughout Africa.	***[61]***
Congo	Broilers and backyard	Over the past 20 years, outbreaks and associated mortality in chickens related to NDV infection have been widely documented in various farming systems.	***[62]***
Ethiopia	chickens	The most prevalent viral disease in these birds, Newcastle disease, frequently causes a variety of ailments, including gastrointestinal, neurological system, respiratory system, and non-gastrointestinal problems.	***[63]***

Humans are susceptible to infection, which may result in reddening, lacrimation, edema of the eyelids, subconjunctival hemorrhage, and conjunctivitis **[64]**. Almost all wild and domestic bird species are susceptible to NDV infection, but chicken is the most susceptible domestic bird species to infection. In contrast to the majority of paramyxoviruses, NDV is heat-stable. It lasts at least six months at -20°C and up to four months at 4°C in the bone marrow and muscle of butchered chicken. The contagious virus may also persist in eggs from infected chickens for over a year at 4°C and months at ambient temperature **[14]**. The dry season has a higher prevalence of ND than the wet season. Due to the high frequency of ND during the dry season, human activities and increasing chicken market turnover could cause ND outbreaks **[65]**. Studies on ND found that local and crossbred hens had significantly different disease prevalences. Chicken crosses have had higher prevalences **[66]**.

Newcastle Disease Virus Replication Cycle

Hemagglutination Activity

The sialic acid receptors on erythrocytes' surfaces are the target site of the viral HN protein, allowing NDV and other avian paramyxoviruses to hemagglutinate RBCs **[67]**.

Neuraminidase Activity

The neuraminidase enzyme activity is mediated by the HN protein, which breaks down the sialic acid receptor and prevents viral particles from adhering to themselves and clumping together. The virus can eventually be released from the RBCs due to the enzyme activity.

Cell Fusion and Hemolysis

Paramyxovirus fusion proteins cause hemolysis and fusing of other cells at neutral pH **[68]**. The precursor F0 protein's cleavage into the heterodimer (F1 and F2), coupled with a disulfide bond by the host protease's proteolytic activity, determines the virus' ability to fuse and infect **[69]**.

Virus Replication

The entry of the NDV nucleocapsid complex is mediated by the HN attaching the virus to the receptor on the host cell, after which the F protein fuses the virus to the cell membrane of the host **(**Figure 1**)**. N-linked glycoproteins and cholesterol may be necessary for the virus to enter the body, and endocytosis may be the route through which it is done **[70]**. The host cell's cytoplasm provides the only place where paramyxoviruses may multiply. The negative-sense RNA genome is transcripted into positive sense 5′-capped and 3′-polyadenylated messenger RNAs by the virus's internal RNA-dependent RNA polymerase (L), which is released into the cytoplasm to initiate replication (mRNA).

Downstream genes' transcription levels are reduced due to the genes' sequential and polar transcription, which occurs from 5′ to 3′ of the coding sequence **[71]**. As a result, more of the 5′ structural proteins than the 3′ polymerase protein is generated. The mRNA is translated into viral proteins, followed by the negative sense genome replication. This creates the antigenomic RNA that acts as a template for synthesizing the genomic RNA **[72]**. Some NDV strains also produce the precursor HN0, the HN protein **[73]**. It takes posttranslational cleavage to remove the 45 amino acid part of the carboxy-terminal. Trypsin and other proteases (elastase, chymotrypsin, and thermolysin) can break the HN0 but not the fusion protein of viruses with low pathogenicity

[74]. The infected cell's membrane receives and incorporates the HN proteins created inside it. Virus particles emerge from the cell surface due to the nucleocapsid and viral RNA alignment adjacent to the cell membrane areas harboring the viral glycoproteins **[75]**.

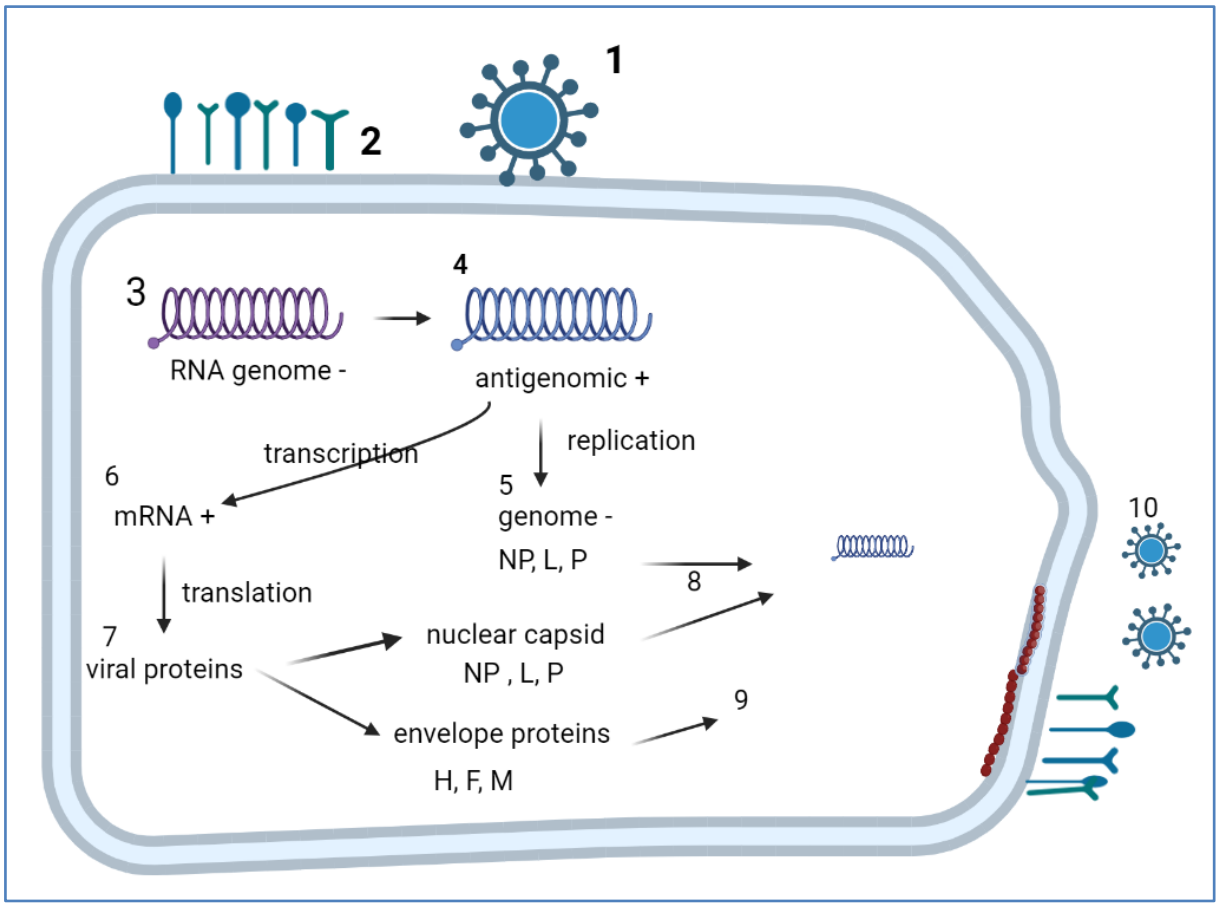

Figure 1. Newcastle disease cell cycle. 1) binding of HN protein; 2) fusion of F-protein; 3) release of RNA genome into the cytoplasm; 4) negative genome is charged to antigenomic (positive); 5) replication of antigenomic (+) to negative (-) genome; 6) transcription of RNA genome to mRNA; 7) translation of mRNA to viral proteins; 8) nuclear capsid proteins closed to the cell membrane; 9) envelope proteins closed to the cell membrane; 10) formation of new virus progeny.

Newcastle Disease Virus Pathogenicity and Lesions

NDV strains differ significantly in virulence depending on the host. Ducks may get infections and exhibit few clinical manifestations, even with fatal strains for chickens, while hens are very vulnerable **[76]**. Although dose, delivery method, bird age, and environmental factors also impact the pathogenicity of ND in chickens, the virus strain has the most impact. In general, the sickness is more severe in younger chickens. Sudden death may occur in young chickens infected with virulent viruses without significant clinical signs, while older birds may show a more prolonged illness with evident clinical signs. Breed or genetic stock impacts chickens' innate immune responses and disease susceptibility **[77]**. Mild to severe cases of the infection are possible. Exotic ND is a particularly lethal and contagious illness that causes many birds to die abruptly without displaying symptoms **[50]**.

The gross lesions are not pathognomonic. Hence, a preliminary diagnosis must be made after examining multiple birds, and the definitive diagnosis is made after isolation and identification of the virus. However, velogenic NDV infections result in significant gross lesions **[78]**.

ND infection may cause lesions such as periorbital region or entire head swelling, edema of the neck's peritracheal tissue, especially at the thoracic inlet, congestion, and occasionally hemorrhage in the lower pharynx and tracheal mucosae. Also, edema in the esophagus, respiratory lymphoid tissue ulcerations, necrosis, and hemorrhages may occur. Mucosal bleeding and significant tracheal congestion are symptoms of gross pathologic alterations to the respiratory tract. Air sac thickening with catarrhal or caseous exudates is frequently associated with secondary bacterial infections, and airsacculitis may be observed with relatively moderate strains **[14, 79]**. A few tiny ecchymoses are concentrated near the glands' openings on the proventriculus' mucosa. The proventriculus, ceca, and small and large intestines are frequently among the infected organs, with the most noticeable lesions due to necrosis of the intestinal wall or lymphoid tissue, e.g., cecal tonsils and Peyer's patches, which become edematous and hemorrhagic. Peyer's patches' ulceration/necrosis suggests ND, although these lesions are not pathognomonic. Edema, hemorrhages, or degeneration of the ovaries may occur. Birds may also have hemorrhages of the bursa of Fabricius and thymus **[80]**, which is less evident in older birds (**Figure 2**). The spleen may sometimes be swollen, friable, dark red, or speckled. Pulmonary edema and pancreatic necrosis may be seen infrequently **[79, 81, 82]**. Mild neural lesions such as focal to diffuse encephalitis with encephalomalacia, congested liver, and enlarged, congested kidneys may occur in the brain **[79]**.

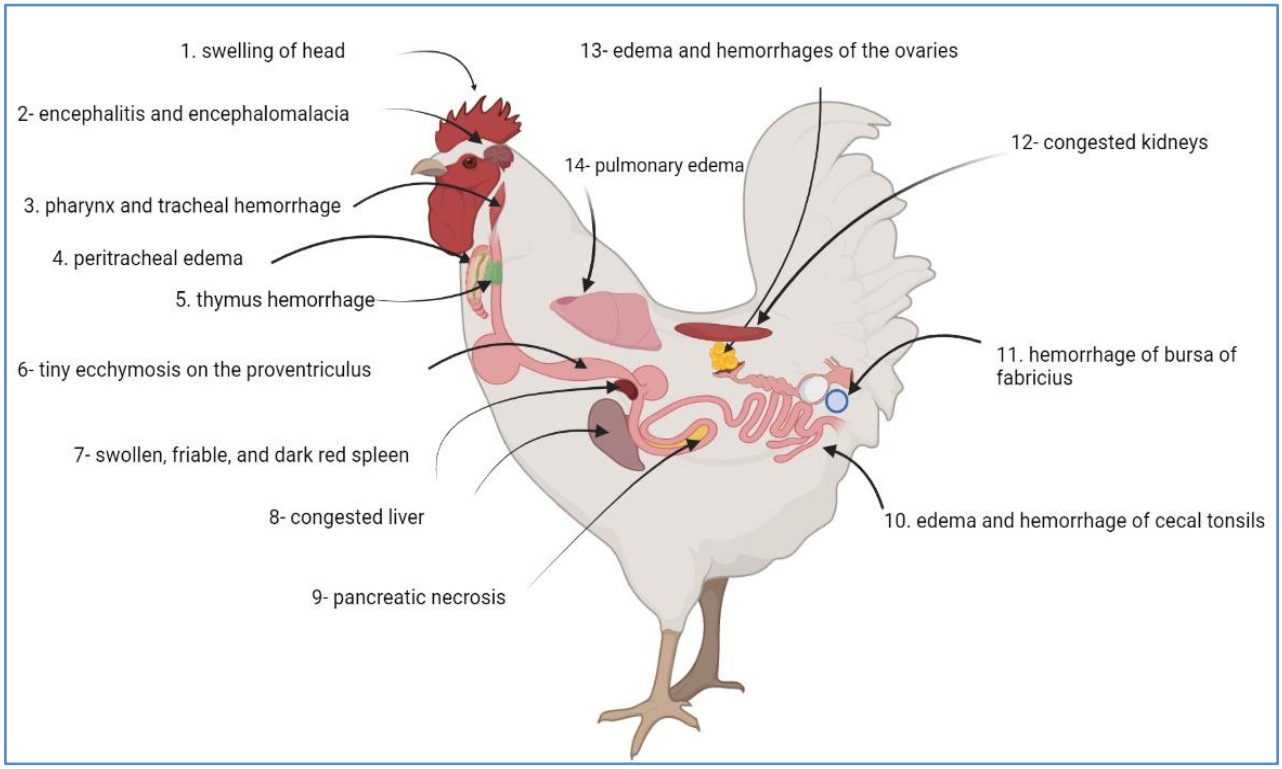

Figure 2. Newcastle disease lesions in chickens.

Newcastle Disease Virus Strains

Most laboratories currently use phylogenetic analysis of genomic sequences as the de facto method for characterizing NDV strains. At least three different genome sizes of APMV-1 isolates exist, which are 15186, 15192, and 15198 **[83]**, observing the rule of six **[72]**. While the initial analysis concentrated on fragments of the F gene **[84]**, recent efforts compared the whole F or complete genome sequences because it is crucial in

virulence determination **[85]**. The 374 base pair (bp) long partial sequence of the fusion protein around the location where the F0 precursor is cleaved into the F1 and F2 fragments is adequate for the molecular assessment of virulence **[86]**. The genetic diversity is reflected by lineages or genotypes of viruses, which have modest recombination rates but significant antigenic drift over time **[87]**. There are two primary classes of APMV-1 strains: class I and class II **[88]**. Class II viruses were first separated into numerous genotypes representing loNDV and vNDV, while class I viruses are predominantly loNDV found in wild birds **[89]**. A new genotyping approach has been proposed to characterize the present genotypes and detect new emerging genotypes based on the mean evolutionary distance between genetic groups of 10%. This method has been made possible by including more full genomes for study **[90]**. More complete genome sequence data for loNDV from wild birds are now accessible, although most of the available sequence data is for class II viruses, including vaccine and virulent viruses **[91]**. The HN protein has eight predicted lengths (571, 572, 577, 578, 580, 582, 585, and 616 amino acids) **[92]**, and some strains can develop an HN0 precursor that can be activated by being cut off at the carboxy terminus **[91]**. Only one vNDV from class I (APMV/1 domestic fowl/Ireland/IECK90187/1990) occurs **[84]**.

Spread, Carriers, Vectors, and Transmission

NDV horizontal transmission has been extensively studied. Infected birds release NDV in their feces and oropharyngeal secretions **[93]**. Susceptible birds can inhale or consume virus aerosols or contaminated dust to cause infection **[94]**. Chicken infection can result from eating contaminated carcasses or feces **[95]**. Oral infection is demonstrated by vaccinating chickens through drinking water **[96]**. When challenged, immunized hens may sweat vNDV for 6–9 days or till death **[97]**. Unvaccinated exotic birds in the wild or captivity, such as pigeons, parrots, and cormorants, may shed vNDV for an extended period without displaying any symptoms **[98]**. Hatchlings could get the infection from contaminated excrement through eggshell cracks or environmental exposure. Hence, vertical transmission of NDV occurs, and reports of pathogenic NDV isolated from ECE are available **[99]**. Day-old chicks and birds dead in their shells have been recorded **[100]**. Some NDVs may prefer the oviduct, which can be seen directly and indirectly using immunohistochemistry and microscopic lesions **[101]**. vNDV was identified from the albumen, yolk, eggshell, ovary, and all components of the oviduct in non-vaccinated chickens. This confirms vertical transmission if embryos remain alive following incubation and hatching **[102]**.

Although people can be infected and develop conjunctivitis, there is no proof that humans, mammals **[103]**, or insects **[104]** are efficient biological vectors for ND. However, the movement of contaminated fomites is more likely to be a medium for human dissemination **[105]**. In addition, the contact of infected birds with susceptible birds and the use of contaminated poultry goods such as feed, water, clothing, shoes, equipment, feed, and vaccines can cause the virus to spread **[106]**. The introduction of ND in some areas has been attributed to migratory and illegally imported birds **[32]**. Biosecurity is essential to control ND and prevent its spread to a poultry facility or disease-free country. Strict importation and quarantine procedures must be followed to avoid transporting infected birds, bird products, and contaminated equipment from where outbreaks occur **[89]**.

Clinical Signs

The clinical signs of ND range considerably, from extremely high morbidity to carriers with no symptoms. The virulence of the virus, the pathotype, the host species and age, the immunological state, coinfection with other organisms, and environmental conditions are only a few of the variables that affect how severe an infection is **[107, 108]**. Clinical symptoms by themselves do not provide a solid basis for ND diagnosis. Mortality and morbidity are influenced by flock health, environmental factors, and level of immunity **[109]**.

The respiratory system can cause rales, coughing, sneezing, and gasping. Tremors, paralytic legs and wings, torticollis, circling, clonic spasms, and total paralysis are neurological signs that occur in broilers. Greenish diarrhea, depression, lack of appetite, decreased egg production, and increased malformed eggs are additional general signs that might be observed in layers and broiler breeders **[110]**.

Viscerotropic velogenic ND isolates are highly virulent and can result in up to 90% fatality rates. Ruffled feathers, lethargy, lack of appetite, and edema of the conjunctiva are among the early indications of infection in backyard and village chickens. As the illness worsens, birds may have a cyanotic coloring of the head and neck, dyspnea, inflammation, and greenish or white watery diarrhea. Neurologic symptoms, including torticollis, tremors, tonic or clonic spasms, wing or leg paresis or paralysis, and abnormal circling behavior, may also be observed in the later stages of the disease. Egg production drastically decreases, and the eggs are deformed with unusually colored, tough, or thin shells and liquid albumen. Velogenic strains that are viscerotropic induce rapid death with little to no warning. Birds that recover from a severe infection may experience neurologic symptoms and a partial or total halt of egg production **[109]**.

Acute symptoms of the neurological system characterize infections with neurotropic velogenic strains. Along with coughing and other respiratory tract symptoms, sudden changes in mood, inappetence, and a decrease in egg production are observed. Mortality is typically between 10 and 20 percent for adult birds, but it can be more significant for young birds **[14, 111]**.

Mesogenic strains result in respiratory distress symptoms. Wheezing, gurgling, and labored breathing are common symptoms, along with depression, weight loss, and a three-week drop in egg production in layers. The average mortality rate is 10% **[14]**.

Microscopic Lesions

The most frequent microscopic lesions for virulent NDV in chickens and turkeys are 1) necrosis of the eyelid, bursa, cecal tonsils, spleen, thymus, pancreas, thymus, liver, and bone marrow, 2) necrosis and ulceration of gut epithelium with petechial hemorrhage at the proventricular region, 3) hemorrhagic, ulcerative tracheitis, 4) necrotic myocarditis, 5) histiocytic airsacculitis, 6) lymphohistiocytic airsacculitis, 7) gliosis with perivascular cuffing of the medulla, cerebellum, and brain stem, and 8) yolk peritonitis with foamy macrophages in the oviduct's subserosal region. In addition, infections with loNDV in hens and turkeys can result in lymphoplasmacytic tracheitis with deciliation **[112]**, but lesions are uncommon **[113]**.

Diagnosis

It is essential to be able to recognize NDV-infected birds and recognize vaccination viruses apart from pathogenic viruses. Clinical signs and indicators associated with vNDV infections are not pathognomonic, making diagnosis impossible. Many countries do not record infections with loNDV strains since they could be caused by lentogenic or vaccine viruses. LoNDV is frequently detected in seemingly healthy wild birds ***[114]***.

Newcastle Disease Virus Isolation and Identification

Swabs from a living bird's trachea, oropharynx, cloaca, or tissue samples from a dead bird's brain, kidney, liver, or spleen can be used to isolate and identify NDV. The samples are kept frozen until they can be used for viral detection. Swabs should be immersed in a viral transport medium (VTM), and about 100–200 microliters of a sample are injected into a 9- to 11-day-old SPF-ECE at the allantoic sac region after centrifugation at 1,000 g for 10 minutes. Inoculated eggs are kept at 37°C and checked daily for 4–7 days **[115]**.

RNA may be extracted from this fluid and used in molecular procedures. Real-time RT-PCR (RRT-PCR) and other molecular diagnostic procedures are frequently performed for confirmation. Cell culture may be utilized to detect NDV as an alternative to ECE. However, except in some cells, such as chicken embryo kidney cells (CEKs), loNDV will not replicate more than once without adding trypsin to the media. RT-PCR or RRT-PCR tests can be used to directly detect viral RNA that has been extracted from swab samples or tissues. Some RRT-PCR assays can identify vNDV from loNDV and distinguish APMV-1 **[116]**.

Serology

Serology is typically not helpful in diagnosing ND since current serologic techniques cannot distinguish between antibodies produced by infection with vNDV, loNDV, or those made by immunization with inactivated or live vaccines. Serology is usually used as a diagnostic tool to assess a vaccination program's efficacy **[117]**. Nonvaccinating countries are in a position to use serology to confirm NDV exposure in nonvaccinating nations. Rising HI titers and clinical symptoms that cannot be assigned to revaccination in well-managed flocks imply that exposure occurred.

Enzyme-linked immunosorbent assays (ELISAs) and HI assays are frequently utilized to identify and measure antibodies **[118]**. Hemagglutination inhibition assays, better than the more time-consuming viral neutralization (VN) assays, are commonly used to assess antibody response following vaccination **[119]**. Commercial ELISA kits are occasionally used to evaluate a flock's immunization consistency. However, they might not correlate well with protection since they do not explicitly detect neutralizing antibodies **[29]**. Although ELISA typically detects antibodies against all NDV proteins and HI assays only detect antibodies to HN, the techniques have some association. ELISAs use a primary monoclonal antibody (mAb) to NDV and a secondary, species-specific mAb; hence, they are often specific for the host species. Use of sera from species other than chickens for the HI test should be done with caution as they may result in false positive results ($\leq$ 1:8) from nonspecific chicken RBCs' agglutination **[120]**. Before being tested, such agglutination can be eliminated by heating it to

56°C for 30 minutes. The OIE-approved protocol is a suitable guide to prevent variations in results **[121]**. Titers of 1:16 or greater when utilizing four HA antigen units are regarded as positive. When eight HA units are used, the cutoff equals or exceeds 1:8 **[3]**.

Differential Diagnosis

ND can be mistaken for diseases with similar clinical presentations, including aspergillosis, chicken cholera, highly pathogenic avian influenza, infectious bronchitis, infectious laryngotracheitis, and mycoplasmosis. Some avian viruses, such as avian paramyxovirus, avian influenza virus, and egg drop syndrome-causing adenovirus, can hemagglutinate chicken RBCs. NDV polyclonal antibodies enable fast confirmation of APMV-1. Appropriate positive and negative controls must be employed for the HI assays for correct diagnosis to avoid cross-reactions with other APMVs **[107]**.

Molecular Methods for ND Diagnosis

Laboratory diagnosis must be made quickly and accurately if ND is to be controlled. Due to advanced molecular techniques, results can be obtained soon after the laboratory receives the samples, usually within a few hours, compared to the conventional methods, which take 2–14 days to isolate and identify the virus. Speedy confirmation is necessary to lessen the economic consequences on a farm while waiting for the results of an investigation. Regulatory authorities' measures directly impact readiness to report concerns and submit samples to the lab **[75]**. Molecular approaches may yield faster results if the absence of clinical indications does not appropriately identify infected birds. Virus isolation is required for flocks that have been immunized or infected with loNDV. Remember that randomly evaluating healthy birds is less effective than sampling clinically unwell or dead birds from flocks that have received vaccinations **[122]**. The OIE suggests molecular assays should identify NDV and distinguish vaccine or loNDV strains from vNDV isolates. The genetic diversity of the fusion gene of various NDV genotypes, specifically the cleavage site in F0, is a common cause of issues with all molecular assays. No verified test can detect and characterize all class I and II viruses globally.

Reverse transcription (RT) is a crucial first step in most molecular procedures, followed by a PCR to create a DNA copy of the RNA genome. RRT-PCR methods, which use fluorescent probes to observe amplification in real-time, have primarily supplanted conventional RT-PCR experiments. Real-time RT-PCRs can be automatized, and they are suitable for high-throughput applications. While most class II genotype viruses were demonstrated to be detectable by primers and probes targeting the M gene, it was later discovered that they frequently missed class I viruses **[123]**. Primers and probes were designed to target the L gene to make the assay conditions and primer/probe sequences compatible with the M gene RT-PCR, permitting a multiplex RRT-PCR to identify class I viruses **[124]**. When one lab's M gene RRT-PCR procedure could not discover some isolates, another lab used a shorter probe by applying locked nucleic acids to preserve probe stability and boost effectiveness **[125]**. Multiplex RRT-PCR can detect most NDVs but not their virulence. As a result, it cannot be used to confirm a vNDV infection; instead, it can only be used to rule out ND as a potential source

of an outbreak. The NDV isolate could be identified, and its pathotype determined using a real-time RT-PCR assay targeting the fusion gene at the cleavage site **[126]**.

Prevention and Control

Immunization and hygiene are the two primary ways to prevent ND. These precautions are usually required, especially in semi-intensive systems for managing ND, where birds are confined inside a gated yard or building. Cleaning, disinfecting, preventing access to wild birds, and maintaining the hygiene of farm employees are all examples of hygiene. Vaccination, in conjunction with sensible hygiene practices, is the best method for controlling ND. Vaccination protects the flocks against infection and replication of the virus. Immunization against ND typically protects the bird from the disease's severe effects; however, viral replication and shedding may still occur **[127]**.

Vaccination

At the time of the virus's apparent emergence, vaccination was first used as an inactivated virus, and it was thought to be a potential method for controlling ND. Then, an attenuated live vaccine, strain H, was created following the epidemic in England in 1933. Later, Hitchner B1 (HB1) and La Sota, two low-virulence USA isolates, were the most widely used veterinary vaccines in the entire globe **[128]**. A viral disease vaccination aims to provoke an immune reaction against the virus without causing the sickness. The simplest way is to isolate and kill the virus and use it to immunize the bird. This kind of vaccine is an inactivated type. A different strategy is to choose a naturally existing virus that is not sufficiently aggressive to cause severe illness and then use it to infect the birds. This kind of vaccine is alive. The second strategy can be developed further by starting with a naturally occurring, non-virulent virus and choosing a desirable clone from the virus population, such as one that is heat- or vaccine-tolerant. This vaccine is a live, cloned one. Finally, it is feasible to create a vaccine genetically, for instance, by extracting a portion of a virus's genetic coding for a surface antigen and putting it into another, unrelated virus to create a recombinant vaccine **[109]**.

Live lentogenic, live mesogenic, and inactivated vaccines are the three categories of ND vaccines. All these immunization strategies have been used for ND. Field viruses, proven to be low pathogenic to poultry but induce a sufficient immune response, are used to make live lentogenic vaccines. HB1, La Sota, F, and several asymptomatic enteric-type viruses—usually based on the V4 or Ulster 2C viruses—are typical vaccine strains. However, manufacturers have regularly applied selection pressure to these viruses to increase their immunogenicity or make them usable for a specific application strategy **[18]**.

It is possible to create inactivated vaccines by propagating an ND virus in eggs and then inactivating the infectious allantoic fluid with a substance like formalin or beta-propiolactone. The inactivated virus is typically given with an adjuvant, like mineral oil, to increase its immunogenicity. The vaccine must be administered individually to each bird needing immunization because it can no longer spread or multiply. About 0.3 or 0.5 mL per bird is typically injected I.M. into the muscles of the thigh or breast or S.C. into the neck. Very high antibody levels are produced against NDV by inactivated vaccinations, which defend against the virulent virus

[129]. In intensive chicken production, in the absence of initial live-virus vaccination, inactivated vaccines are often administered following a first priming immunization **[63]**. Although inactivated vaccines provide adequate protection, they are relatively expensive and have a slight danger of unintentional self-injection for the user. In addition, inactivated vaccinations are somewhat heat-sensitive, although they are significantly less so than traditional live vaccines, making transportation to villages more practical **[130]**.

Because live vaccines may multiply in the host, they vary from inactivated vaccinations, which have benefits and drawbacks **[131]**. It is advantageous because the vaccine virus can transfer from one bird to another, eliminating the need to vaccinate every bird separately. However, since a live virus infection is involved, this could lead to clinical symptoms due to the vaccine virus's inherent virulence or by aggravating other possible pathogens, particularly in the respiratory system. As a result, the particular vaccine strain used and the existence of concurrent infection affect the intensity of this reaction. Live vaccinations have the added benefit of being easier to administer than inactivated vaccines because they can be injected or dropped into drinking water **[132]**.

Treatment

Since vNDV has no known cure, it is typically necessary to slaughter all sick birds to stop an outbreak. Exotic or endangered bird species in an outbreak region may occasionally be quarantined for one month to undergo vNDV testing **[133]**. Controlling secondary bacterial infections, especially Gram-negative bacteria, is the mainstay of treatment for infections with loNDV **[3]**.

Recent research suggests that the antiparasitic drug NTZ (nitazoxanide), a member of the thiazide family, may inhibit the NDV. As a result, they observed the compound's effects on chicken fibroblast cells (DF-1), chicken eggs that have been fertilized, and 14-day-old chickens **[134]**.

The herbs and plant extracts have been reported to exert antiviral action against NDV in ovo, such as 1) green tea leaf extract-derived green silver nanoparticles **[135]**, 2) ethanolic and acetonic extracts of Iresine herbstii **[136]**, and 3) the herbal combination of nilavembu, fenugreek, coriander, turmeric, and garlic **[137]**. In addition, the action of onion and garlic extracts may block the virus's ability to attach to cells in ECEs **[138]**. Inoculated ECEs with NDV were treated with 3α-friedelanol triterpenoids from Synadenium glaucescens (milk bush plant) leaves, eliminating the viral load and preserving embryo viability **[139]**.

Conclusions

Although discovered about a century ago, ND is still devastating and causes substantial economic losses in Asia, the Middle East, and Africa. Sporadic outbreaks also occur in Europe. vNDV is endemic in Iraq and causes high morbidity and mortality rates in the affected farms. There is no approved therapy; vaccination is the only way to protect chickens in endemic areas. Several studies suggest using antiparasitic compounds and plant extracts to treat ND. However, these compounds' molecular mechanisms of action must be elucidated before they can be approved to treat this viral disease.

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Conflict of Interest

The authors declare no conflicts of interest regarding this manuscript's publication and/or funding.

References

1. Ganar K., Das M., Sinha S. & Kumar S. (2014). Newcastle disease virus: current status and our understanding. *Virus Research*. **184**: 71-81.
2. Absalón A., Cortés-Espinosa D.V., Lucio E., Miller P. & Afonso C. (2019). Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. *Tropical Animal Health and Production*. **51**(5): 1033-1048.
3. Suarez D.L., Patti J. Miller, Guus Koch, Egbert Mundt & Rautenschlein S., *Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections*, in *Diseases of Poultry*, David E. Swayne, et al., Editors. 2020, John Wiley & Sons, Inc.: Hoboken, NJ 07030, USA. p. 109-166.
4. Hines N.L. & Miller C.L. (2012). Avian paramyxovirus serotype-1: a review of disease distribution, clinical symptoms, and laboratory diagnostics. *Veterinary Medicine International*. **2012**.
5. Deka P., Nath M.K., Das S., Das B.C., Phukan A., Lahkar D., Bora B., Shokeen K., Kumar A. & Deka P. (2022). A study of risk factors associated with Newcastle disease and molecular characterization of genotype XIII Newcastle disease virus in backyard and commercial poultry in Assam, India. *Research in Veterinary Science*. **150**: 122-130.
6. Mngumi E.B., Mpenda F.N. & Buza J. (2022). Epidemiology of Newcastle disease in poultry in Africa: systematic review and meta-analysis. *Tropical Animal Health and Production*. **54**(4): 214.
7. Abozaid K.G. & Abdel-Moneim A.S. (2022). Epidemiological surveillance of Newcastle disease virus in Egypt—A 6-year cohort study. *Tropical Animal Health and Production*. **54**(4): 243.
8. Haque M., Hossain M., Islam M., Zinnah M., Khan M. & Islam M. (2010). Isolation and Detection of Newcastle disease virus from field outbreaks in Broiler and Layer chickens by Reverse transcription Polymerase chain reaction. *Bangladesh Journal of Veterinary Medicine*. **8**(2): 87-92.
9. Bechtoldt M.N., De Dreu C.K., Nijstad B.A. & Choi H.-S. (2010). Motivated information processing, social tuning, and group creativity. *Journal of Personality and Social Psychology*. **99**(4): 622.
10. Numan M., Zahoor M., Khan H. & Siddique M. (2005). Serologic status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts. *Pakistan Veterinary Journal*. **25**(2): 55.
11. Mazengia H. (2012). Review on major viral diseases of chickens reported in Ethiopia. *Journal of Infectious Diseases and Immunity*. **4**(1): 1-9.
12. Abolnik C., Phiri T., Peyrot B., de Beer R., Snyman A., Roberts D., Ludynia K., Jordaan F., Maartens M. & Ismail Z. (2023). The Molecular Epidemiology of Clade 2.3. 4.4 B H5N1 High Pathogenicity Avian Influenza in Southern Africa, 2021–2022. *Viruses*. **15**(6): 1383.
13. Oberländer B., Failing K., Jüngst C.M., Neuhaus N., Lierz M. & Möller Palau-Ribes F. (2020). Evaluation of Newcastle Disease antibody titers in backyard poultry in Germany with a vaccination interval of twelve weeks. *Plos One*. **15**(8): e0238068.
14. Abdisa T. & Tagesu T. (2017). Review on Newcastle disease of poultry and its public health importance. *J. Vet. Sci. Technol*. **8**(3): 441.
15. Prajna N.V., Lalitha P., Chen C., Zhong L., Lietman T.M., Doan T. & Seitzman G.D. (2022). Acute keratoconjunctivitis resulting from coinfection with avian Newcastle virus and human adenovirus. *Cornea*. **41**(5): 630-631.
16. Alexander D. (2000). Newcastle disease and other avian paramyxoviruses. *Revue Scientifique et Technique-Office International des Epizooties*. **19**(2): 443-455.
17. Nolen R.S. (2003). Emergency declared: exotic Newcastle disease found in commercial poultry farms. *Journal of the American Veterinary Medical Association*. **222**(4): 411-411.

18. Getabalew M., Alemneh T., Akeberegn D., Getahun D. & Zewdie D. (2019). epidemiology, Diagnosis & Prevention of Newcastle disease in poultry. *American Journal of Biomedical Science and Research*. **16**: 50-9.
19. Hossain I., Parvin R., Rahman M.M., Begum J.A., Chowdhury E.H., Islam M.R., Diel D.G. & Nooruzzaman M. (2023). Comparative pathogenicity of a genotype XXI. 1.2 pigeon Newcastle disease virus isolate in pigeons and chickens. *Microbial Pathogenesis*. **178**: 106068.
20. Masoud F., Mahmood M.S., Abbas R.Z., Cheema H.M.N., Rafique A., Ali S., Aslam R. & Khan M.K. (2023). Engineered Newcastle disease virus expressing the haemagglutinin protein of H9N2 confers protection against challenge infections in chickens. *Process Biochemistry*. **130**: 281-290.
21. Catroxo M., Martins A., Petrella S., Curi N. & Melo N. (2011). Research of viral agent in free-living pigeon feces (Columba livia) in the city of São Paulo, SP, Brazil, for transmission electron microscopy. *International Journal of Morphology*. **29**(2): 628-635.
22. Wu J., Lu R., Wang J., Su J., Gu C., Xie Q., Zhu H., Xiao J. & Liu W. (2023). Establishment of reverse genetics for genotype VII Newcastle disease virus and altering the cell tropism by inserting TMPRSS2 into the viral genome. *Virus Genes*. 1-10.
23. Jadhav A., Zhao L., Liu W., Ding C., Nair V., Ramos-Onsins S.E. & Ferretti L. (2020). Genomic diversity and evolution of quasispecies in Newcastle disease virus infections. *Viruses*. **12**(11): 1305.
24. Ahmad-Raus R., Ali A.M., Tan W.S., Salleh H.M., Eshaghi M. & Yusoff K. (2009). Localization of the antigenic sites of Newcastle disease virus nucleocapsid using a panel of monoclonal antibodies. *Research in Veterinary Science*. **86**(1): 174-182.
25. Linde A.-M., Munir M., Zohari S., Ståhl K., Baule C., Renström L. & Berg M. (2010). Complete genome characterisation of a Newcastle disease virus isolated during an outbreak in Sweden in 1997. *Virus Genes*. **41**(2): 165-173.
26. Park M.-S., Shaw M.L., Munoz-Jordan J., Cros J.F., Nakaya T., Bouvier N., Palese P., García-Sastre A. & Basler C.F. (2003). Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins. *Journal of Virology*. **77**(2): 1501-1511.
27. Duan Z., Zhang Q., Liu M. & Hu Z. (2023). Multifunctionality of matrix protein in the replication and pathogenesis of Newcastle disease virus: A review. *International Journal of Biological Macromolecules*. 126089.
28. Kho C., Tan W., Tey B. & Yusoff K. (2004). Regions on nucleocapsid protein of Newcastle disease virus that interact with its phosphoprotein. *Archives of Virology*. **149**(5): 997-1005.
29. Mao Q., Ma S., Schrickel P.L., Zhao P., Wang J., Zhang Y., Li S. & Wang C. (2022). Review detection of Newcastle disease virus. *Frontiers in Veterinary Science*. **9**: 936251.
30. Moura V., Susta L., Cardenas-Garcia S., Stanton J., Miller P., Afonso C. & Brown C. (2016). Neuropathogenic capacity of lentogenic, mesogenic, and velogenic Newcastle disease virus strains in day-old chickens. *Veterinary Pathology*. **53**(1): 53-64.
31. Roohani K., Tan S.W., Yeap S.K., Ideris A., Bejo M.H. & Omar A.R. (2015). Characterisation of genotype VII Newcastle disease virus (NDV) isolated from NDV vaccinated chickens, and the efficacy of LaSota and recombinant genotype VII vaccines against challenge with velogenic NDV. *Journal of Veterinary Science*. **16**(4): 447.
32. 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.
33. Gilchrist P. (2005). Involvement of free-flying wild birds in the spread of the viruses of avian influenza, Newcastle disease and infectious bursal disease from poultry products to commercial poultry. *World's Poultry Science Journal*. **61**(2): 198-214.
34. Kuiken T., Leighton F.A., Wobeser G., Danesik K.L., Riva J. & Heckert R.A. (1998). An epidemic of Newcastle disease in double-crested cormorants from Saskatchewan. *Journal of Wildlife Diseases*. **34**(3): 457-471.
35. Shahzad M., Mubashir H., Umar F., Qaiser J., Mehwesh A., Kalsoom B., Jafar K., Sultan A., Kil Y.K. & Muhammad A. (2012). Quantification of antibodies against poultry haemagglutinating viruses by haemagglutination inhibition test in Lahore. *African Journal of Microbiology Research*. **6**(21): 4614-4619.
36. Leid J.G., Ditto A.J., Knapp A., Shah P.N., Wright B.D., Blust R., Christensen L., Clemons C., Wilber J. & Young G.W. (2012). In vitro antimicrobial studies of silver carbene complexes: activity of free and

nanoparticle carbene formulations against clinical isolates of pathogenic bacteria. *Journal of Antimicrobial Chemotherapy*. **67**(1): 138-148.
37. Karthik L., Kumar G., Keswani T., Bhattacharyya A., Chandar S.S. & Bhaskara Rao K. (2014). Protease inhibitors from marine actinobacteria as a potential source for antimalarial compound. *PloS One*. **9**(3): e90972.
38. Abolnik C. (2017). History of newcastle disease in South Africa. *Onderstepoort Journal of Veterinary Research*. **84**(1): 1-7.
39. Putri D.D., Poetri O.N., Candra A.A. & Soejoedono R.D. (2022). Production of hyperimmune serum against genotype VII Newcastle disease virus in rabbits with several applications. *Journal of Advanced Veterinary and Animal Research*. **9**(2): 211.
40. 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. *Virology Journal*. **8**(1): 1-11.
41. 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. *Journal of Clinical Microbiology*. **40**(10): 3826-3830.
42. Alders R.G. (2014). Making Newcastle disease vaccines available at village level. *Veterinary Record*. **174**(20): 502-03.
43. Leslie J. (2000). Newcastle disease: outbreak losses and control policy costs. *Veterinary Record*. **146**(21): 603-606.
44. Al-Shammari A.M., Hamad M.A., AL-Mudhafar M.A., Raad K. & Ahmed A. (2020). Clinical, molecular and cytopathological characterization of a Newcastle disease virus from an outbreak in Baghdad, Iraq. *Veterinary Medicine and Science*. **6**(3): 477-484.
45. Alazawy A.K. & Al Ajeeli K.S. (2020). Isolation and molecular identification of wild Newcastle disease virus isolated from broiler farms of Diyala Province, Iraq. *Veterinary World*. **13**(1): 33.
46. Dyary H.O., Husseiun R.S., Saeed N.M. & Rashid P.M.A. (2023). Determination of median embryo lethal dose for a velogenic Newcastle disease virus isolated in Sulaimani/Iraq. *Basrah Journal of Veterinary Research*. **22**(4): 1-14.
47. Shadood H.M. & Najem H.A. (2021). Clinical, pathological and molecular study of newcastledisease in layers farms in Basarh, Iraq. *Basrah Journal of Veterinary Research*. **20**(1): 105-121.
48. Al-Murshedy N.A., Al-Zubaidi H.J. & Ghaithsabah Tuaimah Alabedi A. (2023). Histopathological and immunohistochemical study of Newcastle disease in chicken in Al-Najaf Province. *Journal of Survey in Fisheries Sciences*. **10**(3S): 14-25.
49. Sharif Razavian A., Azizpour H., Sullivan J. & Carlsson S. *CNN features off-the-shelf: an astounding baseline for recognition*. in *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition Workshops*. 2014.
50. Ashraf A. & Shah M. (2014). Newcastle disease: present status and future challenges for developing countries. *African Journal of Microbiology Research*. **8**(5): 411-416.
51. Hussain J., Rabbani I., Aslam S. & Ahmad H. (2015). An overview of poultry industry in Pakistan. *World's Poultry Science Journal*. **71**(4): 689-700.
52. Khatun M., Islam I., Ershaduzzaman M., Islam H., Yasmin S., Hossen A. & Hasan M. (2018). Economic impact of newcastle disease on village chickens-a case of Bangladesh. *Journal of Economics and Business*. **1**: 358-367.
53. Spickler A. (2016). Newcastle disease. *The Center for Food Security and Public Health*.
54. Aldous E. & Alexander D. (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Avian Pathology*. **30**(2): 117-128.
55. BAYİR T. & GÜRCAN S. (2022). Retrospective investigation of Newcastle disease reported in Turkey 5 between 2017-2019. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*.
56. Mehrabadi M., Ghalyanchilangeroudi A., Tehrani F., Hajloo S.A., Bashashati M., Bahonar A., Pourjafar H. & Ansari F. (2022). Assessing the economic burden of multi-causal respiratory diseases in broiler farms in Iran. *Tropical Animal Health and Production*. **54**(2): 1-7.
57. Alsahami A., Ideris A., Omar A., Ramanoon S.Z. & Sadiq M.B. (2018). Seroprevalence of Newcastle disease virus in backyard chickens and herd-level risk factors of Newcastle disease in poultry farms in Oman. *International Journal of Veterinary Science and Medicine*. **6**(2): 186-191.
58. Lawal J., El-Yuguda A. & Ibrahim U. (2016). Efficacy of feed coated Newcastle disease i2 vaccine in village chickens in Gombe State, Nigeria. *J. Vet. Sci. Technol*. **7**(3): 349.

59. Pchelkina I., Manin T., Kolosov S., Starov S., Andriyasov A., Chvala I., Drygin V., Yu Q., Miller P. & Suarez D. (2013). Characteristics of pigeon paramyxovirus serotype-1 isolates (PPMV-1) from the Russian Federation from 2001 to 2009. *Avian Diseases*. **57**(1): 2-7.
60. Campbell Z.A., Otieno L., Shirima G.M., Marsh T.L. & Palmer G.H. (2019). Drivers of vaccination preferences to protect a low-value livestock resource: Willingness to pay for Newcastle disease vaccines by smallholder households. *Vaccine*. **37**(1): 11-18.
61. Fisher H., *Newcastle disease control in Africa*. 2014: ACIAR.
62. Kampala U., African Union Interafrican Bureau for Animal Resources. 2009, press.
63. Bello M.B., Yusoff K., Ideris A., Hair-Bejo M., Peeters B.P. & Omar A.R. (2018). Diagnostic and vaccination approaches for Newcastle disease virus in poultry: The current and emerging perspectives. *BioMed research international*. **2018**.
64. Munir M., Shabbir M.Z., Yaqub T., Shabbir M.A., Mukhtar N., Khan M.R. & Berg M., Complete genome sequence of a velogenic neurotropic avian paramyxovirus 1 isolated from peacocks (Pavo cristatus) in a wildlife park in Pakistan. 2012, *Journal of Virology*. 86(23):13113.
65. Nega M., Moges F., Mazengia H., Zeleke G. & Tamir S. (2012). Evaluation of I2 thermostable Newcastle disease vaccine on local chickens in selected districts of western Amhara. *Online Journal of Animal and Feed Research*. **2**(3): 244-248.
66. Zeleke A., Sori T., Gelaye E. & Ayelet G. (2005). Newcastle disease in village chickens in the southern and rift valley districts in Ethiopia. *International Journal of Poultry Science*. **4**(7): 507-510.
67. Kim C.-H. (2020). SARS-CoV-2 evolutionary adaptation toward host entry and recognition of receptor O-Acetyl sialylation in virus–host interaction. *International journal of Molecular Sciences*. **21**(12): 4549.
68. Lamb R.A., Paterson R.G. & Jardetzky T.S. (2006). Paramyxovirus membrane fusion: lessons from the F and HN atomic structures. *Virology*. **344**(1): 30-37.
69. Slaughter K.B. & Dutch R.E. (2019). Transmembrane domain dissociation is required for Hendra virus F protein fusogenic activity. *Journal of Virology*. **93**(22): e01069-19.
70. Martín J.J., Holguera J., Sánchez-Felipe L., Villar E. & Muñoz-Barroso I. (2012). Cholesterol dependence of Newcastle disease virus entry. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. **1818**(3): 753-761.
71. Lamb R.A. (2001). Paramyxoviridae: the viruses and their replication. *Fields Virology*.
72. Peeters B., Gruijthuijsen Y., De Leeuw O. & Gielkens A. (2000). Genome replication of Newcastle disease virus: involvement of the rule-of-six. *Archives of Virology*. **145**(9): 1829-1845.
73. Jin J., Zhao J., Ren Y., Zhong Q. & Zhang G. (2016). Contribution of HN protein length diversity to Newcastle disease virus virulence, replication and biological activities. *Scientific Reports*. **6**(1): 36890.
74. Karsunke J., Heiden S., Murr M., Karger A., Franzke K., Mettenleiter T.C. & Römer-Oberdörfer A., *W protein expression by Newcastle disease virus*. *Virus Research*. 2019. 263(2): 207-216.
75. Elbers A., Gorgievski-Duijvesteijn M., Zarafshani K. & Koch G. (2010). To report or not to report: a psychosocial investigation aimed at improving early detection of avian influenza outbreaks. *OIE Revue Scientifique Et Technique*. **29**(3): 435-449.
76. Elbestawy A.R., Ellakany H.F., Abd El-Hamid H.S., Zedan R.E., Gado A.R., Sedeik M.E., Abd El-Hack M.E., Saadeldin I.M., Alowaimer A.N. & Ba-Awadh H.A. (2019). Muscovy ducks infected with velogenic Newcastle disease virus (genotype VIId) act as carriers to infect in-contact chickens. *Poultry Science*. **98**(10): 4441-4448.
77. Schilling M.A., Memari S., Cavanaugh M., Katani R., Deist M.S., Radzio-Basu J., Lamont S.J., Buza J.J. & Kapur V. (2019). Conserved, breed-dependent, and subline-dependent innate immune responses of Fayoumi and Leghorn chicken embryos to Newcastle disease virus infection. *Scientific Reports*. **9**(1): 7209.
78. Sultan H.A., Talaat S., Elfeil W.K., Selim K., Kutkat M.A., Amer S.A. & Choi K.-S. (2020). Protective efficacy of the Newcastle disease virus genotype VII–matched vaccine in commercial layers. *Poultry Science*. **99**(3): 1275-1286.
79. Kabiraj C.K., Mumu T.T., Chowdhury E.H., Islam M.R. & Nooruzzaman M. (2020). Sequential pathology of a genotype XIII Newcastle disease virus from Bangladesh in chickens on experimental infection. *Pathogens*. **9**(7): 539.
80. Etriwati E., Agungpriyono D.R., Setiyaningsih S., Darniati D., Ak D.M., Erwin E. & Handharyani E. (2023). Comparative pathology and immunohistochemistry of Newcastle disease in domestic chicken (*Gallus gallus domesticus*) and Alabio duck (Anas platyrhynchos Borneo). *Open Veterinary Journal*. **13**(4): 433–442-433–442.

81. Usman S., Oladele S., Saidu L., Muhammed M., Umar F., Abubakar A., Saleh A. & Orakpoghenor O. (2020). Clinical and gross-pathological changes in Muscovy ducks and Nigerian local chickens infected with Newcastle disease virus (XIVb strain). *Sokoto Journal of Veterinary Sciences*. **18**(4): 182-190.
82. Shadood H.M. & Najem H.A. (2021). CLINICAL, PATHOLOGICAL AND MOLECULAR STUDY OF NEWCASTLE DISEASE IN LAYERS FARMS IN BASRAH, IRAQ. *Basrah Journal of Veterinary Research*. **20**(1).
83. Czeglédi A., Wehmann E. & Lomniczi B. (2003). On the origins and relationships of Newcastle disease virus vaccine strains Hertfordshire and Mukteswar, and virulent strain Herts' 33. *Avian Pathology*. **32**(3): 269-274.
84. Aldous E., Mynn J., Banks J. & Alexander D. (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. *Avian pathology*. **32**(3): 237-255.
85. Shittu I., Sharma P., Joannis T.M., Volkening J.D., Odaibo G.N., Olaleye D.O., Williams-Coplin D., Solomon P., Abolnik C. & Miller P.J. (2016). Complete genome sequence of a genotype XVII Newcastle disease virus, isolated from an apparently healthy domestic duck in Nigeria. *Genome Announcements*. **4**(1): e01716-15.
86. Kariithi H.M., Ferreira H.L., Welch C.N., Ateya L.O., Apopo A.A., Zoller R., Volkening J.D., Williams-Coplin D., Parris D.J. & Olivier T.L. (2021). Surveillance and Genetic Characterization of Virulent Newcastle Disease Virus Subgenotype V. 3 in Indigenous Chickens from Backyard Poultry Farms and Live Bird Markets in Kenya. *Viruses*. **13**(1): 103.
87. Dimitrov K.M., Ramey A.M., Qiu X., Bahl J. & Afonso C.L. (2016). Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus). *Infection, genetics and evolution*. **39**: 22-34.
88. Czeglédi A., Ujvári D., Somogyi E., Wehmann E., Werner O. & Lomniczi B. (2006). Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Research*. **120**(1-2): 36-48.
89. Dimitrov K.M., Afonso C.L., Yu Q. & Miller P.J. (2017). Newcastle disease vaccines—A solved problem or a continuous challenge? *Veterinary Microbiology*. **206**: 126-136.
90. Diel D.G., da Silva L.H., Liu H., Wang Z., Miller P.J. & Afonso C.L. (2012). 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): 1770-1779.
91. Ren S., Xie X., Wang Y., Tong L., Gao X., Jia Y., Wang H., Fan M., Zhang S. & Xiao S. (2016). Molecular characterization of a Class I Newcastle disease virus strain isolated from a pigeon in China. *Avian Pathology*. **45**(4): 408-417.
92. Zhang Y.-y., Shao M.-y., Yu X.-h., Zhao J. & Zhang G.-z. (2014). Molecular characterization of chicken-derived genotype VIId Newcastle disease virus isolates in China during 2005–2012 reveals a new length in hemagglutinin–neuraminidase. *Infection, Genetics and Evolution*. **21**: 359-366.
93. Liu D., *Manual of security sensitive microbes and toxins*. 2014: CRC Press.
94. Li X., Chai T., Wang Z., Song C., Cao H., Liu J., Zhang X., Wang W., Yao M. & Miao Z. (2009). Occurrence and transmission of Newcastle disease virus aerosol originating from infected chickens under experimental conditions. *Veterinary Microbiology*. **136**(3-4): 226-232.
95. Stromberg Z.R., Johnson J.R., Fairbrother J.M., Kilbourne J., Van Goor A., Curtiss 3rd R. & Mellata M. (2017). Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. *PloS One*. **12**(7): e0180599.
96. Lal M., Zhu C., McClurkan C., Koelle D., Miller P., Afonso C., Donadeu M., Dungu B. & Chen D. (2014). Development of a low-dose fast-dissolving tablet formulation of Newcastle disease vaccine for low-cost backyard poultry immunisation. *Veterinary Record*. **174**(20): 504-504.
97. Kapczynski D.R. & King D.J. (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine*. **23**(26): 3424-3433.
98. Kang Y., Xiang B., Yuan R., Zhao X., Feng M., Gao P., Li Y., Li Y., Ning Z. & Ren T. (2016). Phylogenetic and pathotypic characterization of Newcastle disease viruses circulating in South China and transmission in different birds. *Frontiers in Microbiology*. **7**: 119.

99. Rehan M., Aslam A., Khan M., Abid M., Hussain S., Amber J., Anjum A. & 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.
100. Roy P. & Venugopalan A. (2005). Unexpected Newcastle disease virus in day old commercial chicks and breeder hen. *Comparative Immunology, Microbiology and Infectious Diseases*. **28**(4): 277-285.
101. Bwala D.G., Clift S., Duncan N.M., Bisschop S.P. & Oludayo F.F. (2012). Determination of the distribution of lentogenic vaccine and virulent Newcastle disease virus antigen in the oviduct of SPF and commercial hen using immunohistochemistry. *Research in Veterinary Science*. **93**(1): 520-528.
102. Sá e Silva M., Susta L., Moresco K. & Swayne D.E. (2016). Vaccination of chickens decreased Newcastle disease virus contamination in eggs. *Avian Pathology*. **45**(1): 38-45.
103. Zhao P., Sun L., Sun X., Li S., Zhang W., Pulscher L.A., Chai H. & Xing M. (2017). Newcastle disease virus from domestic mink, China, 2014. *Veterinary Microbiology*. **198**: 104-107.
104. Chakrabarti S., King D.J., Afonso C., Swayne D., Cardona C.J., Kuney D.R. & Gerry A.C. (2007). Detection and isolation of exotic Newcastle disease virus from field-collected flies. *Journal of Medical Entomology*. **44**(5): 840-844.
105. Fan S., Wang T., Gao X., Ying Y., Li X., Li Y., Li Y., Ma J., Sun H. & Chu D. (2015). Phylogenetic analysis of Newcastle disease viruses isolated from wild birds in the Poyang Lake region of China. *Journal of Veterinary Medical Science*. 14-0080.
106. Jensen Handberg K., Ahrens P., Manvell R., Frost K. & Alexander D. (2000). Similarity of avian paramyxovirus serotype 1 isolates of low virulence for chickens obtained from contaminated poultry vaccines and from poultry flocks. *Veterinary Record*. **146**(23): 665-668.
107. Saif Y.M., Swayne D.E., Pantin-Jackwood M.J., Spackman E., Johnson T.J., Day J.M., French D., Gingerich E., Bilgili S. & Jones K. (2020). Emerging diseases and diseases of complex or unknown etiology. *Diseases of Poultry*. 1383-1410.
108. Susta L., Miller P., Afonso C. & Brown C. (2011). Clinicopathological characterization in poultry of three strains of Newcastle disease virus isolated from recent outbreaks. *Veterinary Pathology*. **48**(2): 349-360.
109. Alexander D.J., Bell J.G. & Alders R.G. (2004). A technology review: Newcastle disease, with special emphasis on its effect on village chickens. FAO Animal Production and Health.
110. Kahn C.M., Line S. & Aiello S. (2005). The merck veterinary manual. Merck & Co. *Inc., Whitehouse Station, NJ*.
111. Madadi M.S., Shojaei H. & Ghaniei A. (2016). Assessment of immunity of arian broiler breeders to newcastle disease virus after vaccination by HI: A retrospective study. *International Journal of Basic Sciences & Applied Research*. **5**(4), 248-254.
112. Lam K. (2003). Newcastle Disease virus–induced damage to embryonic tracheae and red blood cells. *Avian Diseases*. **47**(1): 197-202.
113. Brown C.C., Sullivan L., Dufour-Zavala L., Kulkarni A., Williams S., Susta L., Zhang J. & Sellers H. (2013). Comparing presence of avian paramyxovirus-1 through immunohistochemistry in tracheas of experimentally and naturally infected chickens. *Avian Diseases*. **57**(1): 36-40.
114. Van Borm S., Rizotto L.S., Ullmann L.S., Scagion G.P., Malossi C.D., Simão R.M., Araújo Jr J.P., Cordeiro I.M., Keid L.B. & Oliveira T.M.F.S. (2016). Complete genome sequence of a vaccinal Newcastle disease virus strain isolated from an owl (Rhinoptynx clamator). *Genome Announcements*. **4**(6): e01243-16.
115. Ashraf A., Ashraf M.M., Rafiqe A., Aslam B., Galani S., Zafar S., Asad F., Asghar R.D., Akram S. & Ahmed H. (2017). In vivo antiviral potential of Glycyrrhiza glabra extract against Newcastle disease virus. *Pakistan Journal of Pharmaceutical Sciences*. **30**.
116. Laamiri N., Fällgren P., Zohari S., Ben Ali J., Ghram A., Leijon M. & Hmila I. (2016). Accurate detection of avian respiratory viruses by use of multiplex PCR-based luminex suspension microarray assay. *Journal of Clinical Microbiology*. **54**(11): 2716-2725.
117. Ramey A.M., Goraichuk I.V., Hicks J.T., Dimitrov K.M., Poulson R.L., Stallknecht D.E., Bahl J. & Afonso C.L. (2017). Assessment of contemporary genetic diversity and inter-taxa/inter-region exchange of avian paramyxovirus serotype 1 in wild birds sampled in North America. *Virology Journal*. **14**(1): 1-12.
118. Chaka H., Thompson P.N., Goutard F. & Grosbois V. (2015). Evaluation of enzyme-linked immunosorbent assays and a haemagglutination inhibition tests for the detection of antibodies to Newcastle disease virus in village chickens using a Bayesian approach. *Preventive Veterinary Medicine*. **119**(1-2): 21-30.

119. Thampaisarn R., Bui V.N., Trinh D.Q., Nagai M., Mizutani T., Omatsu T., Katayama Y., Gronsang D., Le D.H. & Ogawa H. (2017). Characterization of avian paramyxovirus serotype 14, a novel serotype, isolated from a duck fecal sample in Japan. *Virus Research*. **228**: 46-57.
120. de Sousa R.L.M., Montassier H.J. & Pinto A.A. (2000). Detection and quantification of antibodies to Newcastle disease virus in ostrich and rhea sera using a liquid phase blocking enzyme-linked immunosorbent assay. *Clinical Diagnostic Laboratory Immunology*. **7**(6): 940-944.
121. Stear M.J. (2005). OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees) 5th Edn. Volumes 1 & 2. World Organization for Animal Health.
122. Miller P.J., Dimitrov K.M., Williams-Coplin D., Peterson M.P., Pantin-Jackwood M.J., Swayne D.E., Suarez D.L. & Afonso C.L. (2015). International biological engagement programs facilitate Newcastle disease epidemiological studies. *Frontiers in Public Health*. **3**: 235.
123. Kim L.M., King D.J., Suarez D.L., Wong C.W. & Afonso C.L. (2007). Characterization of class I Newcastle disease virus isolates from Hong Kong live bird markets and detection using real-time reverse transcription-PCR. *Journal of Clinical Microbiology*. **45**(4): 1310-1314.
124. Kim L.M., Suarez D.L. & Afonso C.L. (2008). Detection of a broad range of class I and II Newcastle disease viruses using a multiplex real-time reverse transcription polymerase chain reaction assay. *Journal of Veterinary Diagnostic Investigation*. **20**(4): 414-425.
125. Cattoli G., De Battisti C., Marciano S., Ormelli S., Monne I., Terregino C. & Capua I. (2009). False-negative results of a validated real-time PCR protocol for diagnosis of Newcastle disease due to genetic variability of the matrix gene. *Journal of Clinical Microbiology*. **47**(11): 3791-3792.
126. Fuller C.M., Collins M. & Alexander D. (2009). Development of a real-time reverse-transcription PCR for the detection and simultaneous pathotyping of Newcastle disease virus isolates using a novel probe. *Archives of Virology*. **154**(6): 929-937.
127. Yune N. & Abdela N. (2017). Update on epidemiology, diagnosis and control technique of Newcastle disease. *Journal of Veterinary Science and Technology*. **8**(2): 429.
128. Hannoun C. (2013). The evolving history of influenza viruses and influenza vaccines. *Expert Review of Vaccines*. **12**(9): 1085-1094.
129. Ibrahim Mohamed Adam S., Differentiation between Virulent and Avirulent Newcastle Disease Virus Isolates Using the Cleavage Site Motif of the Fusion Protein. 2012, University of Khartoum.
130. Munir M., Abbas M., Khan M.T., Zohari S. & Berg M. (2012). Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. *Virology Journal*. **9**(1): 1-11.
131. Wang X., Zhou Q., Shen J., Yao J. & Yang X. (2015). Effect of difference doses of Newcastle disease vaccine immunization on growth performance, plasma variables and immune response of broilers. *Journal of Animal science and Biotechnology*. **6**(1): 1-5.
132. Wiseman A. & Berman E.M. (2017). Herd immunity to Newcastle disease virus in broiler flocks in Israel. *Avian Pathology*. **46**(4): 396-402.
133. Krish K.N., The Use of Ventilation Shutdown as an Alternative Depopulation Method for Egg Laying and Turkey Breeder Hens in the Event of a Foreign Animal Disease Outbreak. 2018, North Carolina State University.
134. Antony F., Vashi Y., Morla S., Mohan H. & Kumar S. (2020). Therapeutic potential of Nitazoxanide against Newcastle disease virus: a possible modulation of host cytokines. *Cytokine*. **131**: 155115.
135. Saadh M. (2022). Potent antiviral effect of green synthesis silver nanoparticles on Newcastle disease virus. *Arabian Journal of Chemistry*. **15**(7): 103899.
136. Andleeb R., Ashraf A., Muzammil S., Naz S., Asad F., Ali T., Rafi R., Al-Ghanim K., Al-Misned F. & Ahmed Z. (2020). Analysis of bioactive composites and antiviral activity of Iresine herbstii extracts against Newcastle disease virus in ovo. *Saudi Journal of Biological Sciences*. **27**(1): 335-340.
137. Priya M., Murthy T. & Vijayanand T. (2022). Antiviral effect of herbal mixture (garlic, nilavembu, turmeric, coriander, and fenugreek) against Newcastle disease virus in ovo. *Journal of Applied Poultry Research*. **31**(1): 100229.
138. Harazem R., El Rahman S.A. & El-Kenawy A. (2019). Evaluation of Antiviral Activity of Allium Cepa and Allium Sativum Extracts Against Newcastle Disease Virus. *Alexandria Journal for Veterinary Sciences*. **61**(1).
139. Credo D., Mabiki F., Machumi F., Chacha M., Cornett C. & Styrishave B. (2022). RESEARCH ARTICLE Anti-Newcastle Disease Virus activity of 3β and 3α Friedelanol Triterpenoids from the leaves of Synadenium glaucescens Pax. *Tropical Biomedicine*. **39**(2): 257-264.