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How Do We Use Molecular Knowledge in Diagnosis and Control of Pandemic Avian Viruses?

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Abstract

Pandemic respiratory viruses of poultry have caused significant economic losses in the poultry industry since the 1930s, and molecular and genetic techniques are widely used for diagnosis and control of the infections. Knowledge of changes in the genetic and antigenic characteristics of the pandemic viruses during the time can be really important for human pandemic viruses such as severe acute respiratory syndrome virus-coronavirus-2 and human influenza virus. The use of these techniques plays a vital role in preventing the faulty results and the possible financial losses that may occur due to the limited findings obtained from conventional laboratory tests. In the light of this information, the purpose of this review is to provide an up-to-date assessment of the diagnosis and prevention of major respiratory viruses in poultry and a general and field-oriented scientific perspective that may be useful in the industry. In this context, current approaches for diagnosis and vaccination applications developed using molecular methods based on avian coronavirus infectious bronchitis virus, avian paramyxovirus-1 virus, and avian influenza virus, which are pandemic, are discussed, and solution suggestions for an effective fight are presented.

Keywords: Avian coronavirus, avian influenza virus, avian paramyxovirus-1, diagnosis, molecular techniques

Introduction

The most common infections in poultry are viral respiratory infections. Avian viral respiratory infections can be handled under three main groups:

1. avian coronavirus infectious bronchitis virus (ACoV IBV) infection,
2. avian paramyxovirus-1 (Newcastle disease virus (NDV)) infection, and
3. avian influenza virus (AIV) infection.

Respiratory viral agents basically show a spread starting from the nose and trachea to the lung and air sacs. Therefore, it is challenging to distinguish the respiratory pathological signs and symptoms of these infections. Considering the clinical and pathological findings, we can only make an opinion on the severity, prevalence, and contagiousness of a respiratory disease. Some of the respiratory viral infections such as Newcastle disease (ND) and AI may have a systemic effect throughout the body, while infectious bronchitis (IB) often causes local pathologies (Carlı, 2019). In particular, ACoV IBV pathologies can be observed in a specific way in addition to the respiratory tract, kidney, and oviduct canal depending on the genotype of the ACoV IBV (Blakey et al., 2020; Chacón et al., 2019). Above-mentioned points are extremely important for clinical differential diagnosis, especially when the disease picture is encountered.

In the infectious respiratory disease picture, more than one etiologic agent can be found together (respiratory complex or multifactorial infection), and we can also quickly encounter infections. Which condition will be encountered is closely related to the epidemiology, contamination, virulence, and immune status of the host (Roussan et al., 2008; Sid et al., 2015; Yu et al., 2015).

When confronted with a respiratory infection clinical picture, a prejudice can, of course, be made with anamnesis, clinical, and pathological data. However, considering all possible factors in this picture and starting from primary infections (IB, ND, and AI), laboratory screening of all factors will be relieved in terms of diagnosis in the second stage, if necessary. In this context, the purpose of this review is to evaluate the current molecular genetic approaches in the diagnosis and prevention stages of primary respiratory viral infections

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that cause economic loss in the poultry sector. Moreover, the paper aimed to discuss solutions for existing problems as examples of pandemic virus infections in humans, such as severe acute respiratory syndrome virus-coronavirus-2 (SARS-CoV-2) and influenza viruses.

Major Respiratory Virus Problems and Suggestions for Their Solutions

Avian Coronavirus Infectious Bronchitis Virus Infection

The first pandemic respiratory infection in the poultry should be carefully and specifically evaluated for further understanding of the current human coronavirus pandemic, COVID-19 (Ji et al., 2011; Li et al., 2010). Infectious Bronchitis (IB) is a significant infectious disease when evaluated economically in the poultry industry. This disease, which was first described in the United States in 1930 (Schalk, 1931), is mainly seen in chickens, but it also has been found in pheasants and peacocks (Han et al., 2020; Khataby et al., 2020). Infectious bronchitis, an acute viral infection, begins in the form of respiratory disease and has different effects depending on the virus type. It can cause pathology in the kidneys as well as affect oviducts, leading to a decrease in egg production and quality (Jackwood & de Witt, 2020; Lin & Chen, 2017; Ren et al., 2020). The agent of the disease is ACoV IBV, a *Gammacoronavirus* from the *Coronaviridae* family (Jackwood & de Witt, 2020). Avian coronavirus infectious bronchitis virus, which is an enveloped, spherical, pleomorphic-shaped virus, has thorn-shaped protrusions (spike) on its surface. The ACoV IBV genome consists of a positive-sense, single-stranded RNA, and virions (mature viruses) contain spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins (Bande et al., 2015).

The main problem with ACoV IBV, which is an infection that will never lose its economic importance for poultry businesses worldwide, is that it has many different genotypes, serotypes, and mutants (Lin & Chen, 2017). As with many other RNA viruses, ACoV IBV has a high rate of error during replication and transcription. In contrast, the absence of the proofreading feature of the RNA polymerase allows these errors not to be corrected (Jackwood et al., 2012). Thus, different genotypes of the virus can quickly occur, and even different variants with many different genotypes can be circulated simultaneously in the host. This situation is also called the quasispecies phenomenon, paving the way for different adaptation levels in the host (Montassier, 2010). The formation of new genotypes in the field, the improper use of live vaccines used to combat infection in flocks, and the variation in host immune system pressures faced by the virus in different poultry populations constantly lead to changes in the virus genome. It makes the evaluation of the reflections of genotypic changes that occur in terms of evolutionary biology very complex (Lin & Chen, 2017; Montassier, 2010). In IB, the task of live vaccines, in particular, is to create specific local mucosal immunity in the target organ or tissue of the agent such as in the trachea, the nose, and the lungs and neutralize the colonization of ACoV IBV into these areas by ensuring that mucosal surface anti-ACoV IBV IgAs are present in these tissues at a protective level (Jordan, 2017). However, in cases where ACoV IBV immunization is not performed adequately, non-protective levels of IgA will occur with the vaccine virus in the relevant mucosal areas. In this case, the ACoV IBV strain present in the field (wild-type ACoV IBV) will have the potential to infect the chickens despite being vaccinated and will have the chance to colonize in their respiratory tract with the vaccine strain. Thus, the vaccine and field strain will be found together in the same animal and will have the chance to replicate together in the same cells (Bande et al., 2015; Jackwood et al., 2012). As a result, these spontaneous (errors in the replication and transcription process) and/or misuse of vaccines caused by ACoV IBV genomic variations cause the routine emergence of new variant ACoV IBVs in the field. This also allows viruses to escape from the host immune system, paving the way for the virus to evolve continuously and to produce new antigenic and/or pathotypic variants.

Another situation that causes the formation of new recombinant or mutant viruses is the lack of appropriate protectotype vaccines in the field against IB. Protectotype vaccine means the protection of chickens on the field against the ACoV IBV (de Wit et al., 2017; Smialek et al., 2017). Genotyping of the ACoV IBV strains in the field is required to decide on the protectotype vaccine to be applied to the flock. Exceptionally, although some heterologous genotypes provide cross-protection, it is a current approach to use vaccines of the same genotype as the field strain to solve IB problems in the field. However, another criterion required to have sufficient immunization in the flock is to continue the vaccination with the same genotype until it reaches the level to create immunity at the protective level. For this purpose, the vaccine selected against the field ACoV IBV strain should be administered at appropriate intervals and the optimized doses. Failure to pay attention to these critical features despite vaccinations creates an environment for the development of new recombinant strains or genotypes, combining field strain and different vaccine strains, making it very difficult to combat the newly emerged virus (Bande et al., 2015; Jordan, 2017; Li et al., 2010). In order to determine the genotypes that are problematic in the field and to develop related vaccination strategies, methods based on genotyping are preferred, up-to-date, and globally. Avian coronavirus infectious bronchitis virus reverse transcriptase polymerase chain reaction (RT-PCR) and nucleotide sequencing are performed as the gold standards for the identification of genotypes, causing IB problems in the field. In the ACoV IBV genome, RT-PCR and subsequent sequencing methods are widely used to amplify the highly variable region(s) of the *S1* gene or whole *S1* gene (Kahya et al., 2013; Lin & Chen, 2017; Najafi et al., 2016).

In 2016, a committee composed of well-known scientists on ACoV has reported a nomenclature to re-classify ACoV IBVs based on the whole S1 sequence. In this nomenclature proposal, ACoV IBVs are classified into genetic genotypes (GI), and in that date, ACoV IBVs were grouped into seven genotypes (Table 1). Today, GI 1 is divided into 29 linages, and other 6 genotypes have only one linages each. However, nowadays, we see the number of genotypes and linages can increase due to some newly isolated ACoV IBV S1 mutants with different deletions, recombination in S1 gene could not be placed into this existing classification (Valastro et al., 2016).

In Turkey, IB is one of the most important infections in both layer and broiler flocks, and it can lead to substantial economic losses in the sector. In order to effectively protect against this disease, conducting the genotyping and related analysis of ACoV IBV and appropriate use of protectotype vaccines to be developed in the light of the data obtained play a critical role. Therefore, we conducted a number of large-scale studies supported by Cost FA1207, TUBITAK (Project No: TOVAG-1100914) and Bursa Uludag University Research Fund (Project No: DDP(V)-2016/11) on ACoV IBV genotype profiles of the breeder, layer, and broiler chickens in Turkey. We performed ACoV IBV isolation, detection of ACoV IBV by real-time PCR (qPCR), and genotyping of our isolates by *S1*-gene based-nucleotide sequencing.

Table 1

Classification of Genetic Lineages and Sub-lineages of ACoV IBV Isolates in World in 2016

Genetic Lineage	Sub-lineage	Prototype Genotypes (Country of Origin)
GI	1	M41(USA)-H120(The Netherlands)
GI	2	Holte 393336 (USA)-SDW(China)
GI	3	Gray (USA)-JMK (USA)
GI	4	Holte18988 (USA)-GX2-98 (China)
GI	5	N1/62 (Australia)-V2-02 (Australia)
GI	6	J9 (China)-Vic S (Australia)
GI	7	TP-64 (Taiwan)
GI	8	L613 (USA)-SE17 (USA)
GI	9	Ark99 (USA)-Ark DPI(USA)-Cal99 (USA)
GI	10	K87 (New Zealand)-T6 (New Zealand)
GI	11	IBV/Brasil351/1984-UMFG1141 (Brazil)
GI	12	D207-D274(The Netherlands)
GI	13	UK 4/91 (UK)- Moroccan G/83 (Morocco)
GI	14	B1648 (Belgium)
GI	15	K210(Korea)-02-K620/02 (Korea)
GI	16	Q1-CK/CH/LDL/971-Xindadi (China)
GI	17	AL/6609/98 (USA)-CV-56b (USA)
GI	18	JP8107 (Japan)-53XJ/99II (China)
GI	19	QXIBV (China)-LX4 (China)
GI	20	Qu_mv (Canada)-Qu_16 (Canada)
GI	21	Italian 02 (Italy)-Spain/98/313 (Spain)
GI	22	66GD/98VI-ck/CH/LSH/99I (China)
GI	23	IS/1494/06 (Israel)-Is Var2 (Israel)
GI	24	IBV506 (India)-V13 (India)
GI	25	GA/10216/2010-GA/12274/2012 (USA)
GI	26	NGA/BP61/2007-NGA/N545/2006 (Nigeria)
GI	27	GA/12341/2012 (USA)-Georgia 08 (USA)
GI	28	Ck/CH/LGX/111119-GX-NN-13 (China)
GI	29	10114/14 (China)-10118/14 (China)
GII	1	D1466(The Netherlands)-V1397 (The Netherlands)
GIII	1	N1-88 (Australia)-V1891 (Australia)
GIV	1	AR/6386/97 (USA)-DE/072/92 (USA)
GV	1	018 (Australia)-N103 (Australia)
GVI	1	SDIB781/2012 (China)-TC07-2 (China)
GVII	1	GXNN130021 (China)-10636/16 (China)

Between 2013 and 2017, we had detected IS Var 2 (GI-23) genotype in 95% and Ma5 (GI-1) genotype in 5% of our all breeding types of chickens, and we had not seen 4/91 (GI-13) genotype; our recent data suggest that 4/91 and IS Var 2 genotypes are responsible for IB in equal ratios in layer flocks, and the broiler flocks have higher IS Var 2 genotype (81%) than 4/91 genotype (9%). The reason for the emergence of the 4/91 genotype may possibly be the widespread use of 4/91 genotype-containing vaccines, even if 4/91-like ACoV IBVs were not responsible for IB outbreaks. Unless the improper use of vaccine genotypes that are unmatched with the field genotypes is discontinued, the emergence of new genotypes can be a risk of spread in Turkish poultry flocks. As mentioned above, it is critical to screen the ACoV IBV genotypes in the field to decide the proper vaccine. The commercial PCRs and their modifications such as qPCR containing the primers specific to the genotypes of the IBV have been developed and are on the market and frequently used for quick screening of the genotypes without sequencing in routine poultry diagnostic laboratories. Because these PCR methods for directly detecting genotypes of IBV, instead of the gold standard S1-nucleotide sequencing, produce generally erroneous results so that they are not adequate for making decisions on the genotypes (unpublished data). We should absolutely use the "gold-standard *S1*-nucleotide sequencing method" for reliable identification of ACoV IBV genotypes circulating in our poultry flocks.

Avian Paramyxovirus-1 (Newcastle Disease Virus) Infection

Newcastle disease (ND) is another panzootic or pandemic infection that affects the respiratory, digestive, nervous, and reproductive systems in poultry (Bello et al., 2018b). Newcastle disease virus (NDV) can infect humans and is considered to be a zoonotic agent. It causes conjunctivitis and rhinitis in humans. It is included in the reportable diseases list in the OIE World Organisation for Animal Health (2018). In our country, it is among the notifiable diseases. Strains of avian paramyxovirus 1 from the *Orthoavulavirus* genus, avian orthoavulavirus-1 species, form ND (Afonso et al., 2016). Newcastle disease virus is an enveloped virus and consists of a single-stranded, non-segmented, negative-sense RNA. The NDV genome contains six gene groups that code six structural proteins. These are the nucleocapsid protein (N), phosphoprotein (P), matrix (M), fusion (F), neuraminidase hemagglutinin (HN), and RNA-dependent RNA polymerase (RNAP). Newcastle disease virions are pleomorphic but can be seen around 100-500 nm in diameter or filamentous about 100 nm in diameter. It shows a variation in length (Miller & Koch, 2020).

Neuraminidase hemagglutinin and F protein antigens on the NDV envelope are the structures responsible for the binding of the virus to the host cell. Neuraminidase hemagglutinin proteins ensure the binding of the virus to host cell receptors with the coordinated effect of the F protein. Due to this biological activity, the virus binds to the receptors in the host cells and allows the viral nucleocapsid complex to enter the host cell. In order for the HN protein, which is in direct contact with the host cell, to be attached to the cell during the infection process, it is necessary to change the configuration, that is, the three-dimensional structure. For this change and elongation to occur, it is necessary to bend the localized F protein toward the HN protein and its specific contact. If the F protein changes or the contact to HN is blocked, the HN protein cannot change the configuration, and therefore, NDV cannot be attached to the host cell. Antibodies produced against both (HN and F) antigenic constructs have the function of preventing NDV infection, in other words, blocking the binding of NDV to the host cell. In this context, anti-HN and anti-F antibodies co-neutralize cell binding (Welch et al., 2013).

According to the gene sequencing analyses, NDV strains can be divided into two main classes: Class I and Class II. Class I viruses are low-virulent NDV (loNDV) found mostly in wild birds. Class II viruses can basically be divided into multiple genotypes with loNDV and virulent NDV (vNDV) (Table 2). The average evolutionary distance of 10% between genetic groups is considered a distinctive value

Table 2

Classification of Newcastle Disease Viruses (Avian Paramyxovirus Type-1) According to Fusion Gene Sequencing

Class	Genotype	Sub-genotypes	Information*
I	I	a	loNDVs. Mainly in water birds and also poultry.
		1b	
		1c	
		U	
II	I	Ia	loNDVs. Queensland/V4, I-2, and Ulster 2C/67 strains. Some of them are used as vaccine strains.
		Ib	
		Ic	
	II		vNDVs and loNDVs. Hitchner B1/47, LaSota/46, VG/GA, F-strain, Komarov and Roakin strains. Some of them are used as vaccine strains. Panzootics: 1920-1950s. Regions seen: East Asia
	III		vNDVs. Miyadera/51 and Mukteswar strains Panzootics: 1920-1950s. Regions seen: East Asia
	IV		vNDVs. Herts/33, Italien/44 and Texas GB/48 strains. Panzootics: 1920-1950s. Regions seen: East Asia
	V	Va	vNDVs. Panzootics: 1960s–1970s. Regions seen: North, Central and South Americas.
		Vb	
		Vc	
		Vd	
	VI	VIa	vNDVs and loNDVs. 1980s Panzootics in Pigeons. Region seen: Global distribution.
		VIb	
		VIc	
		VIe	
		VIf	
		VIg	
		VIh	
		VIi	
	VII	VIIb	vNDVs. 1980s Panzootics, Regions seen: Taiwan and Indonesia in the 1980s. Virulent for different avian species including waterfowl. Now global genotype responsible in European Union, Middle East, Asia, southern Africa, and South America's outbreaks.
		VIId	
		VIIe	
		VIIf	
		VIIg	
		VIIh	
		VIIi	
	VIII		vNDVs. Outbreaks 1950-2000. Regions seen: South Africa, Argentina, and East Asia.
	IX		vNDVs. Outbreak between 1930s and 1960s. Regions seen: East Asia.
	X		loNDVs. Waterfowl and shorebirds, Regions seen: North America.
	XI		vNDVs. Regions seen: Madagascar. Possibly derived from Genotype IV NDVs.
	XII		vNDVs. Regions seen: East Asia, South America, and China.
	XIII	XIIIa	vNDVs. Regions seen: Pakistan, Iran, Russia, India, Sweden, and Burundi.
		XIIIb	
	XIV	XIVa	vNDVs. Regions seen: West and Central Africa.
		XIVb	
	XV		vNDVs. Regions seen: China.
	XVI		vNDVs. Regions seen: Central and South America.
	XVII	XVIIa	vNDVs. Regions seen: West and Central Africa.
		XVIIb	
	XVIII	XVIIIa	vNDVs. Regions seen: West and Central Africa.
		XVIIIb	

Note: loNDVs = lentogenic Newcastle disease virus; vNDV = velogenic Newcastle disease virus.
*References: de Almeida et al., 2013; Diel et al., 2012; Dimitrov et al., 2016; Maminiaina et al., 2010; Miller et al., 2015; Rui et al., 2010.

in identifying existing genotypes and new genotypes. In order for this disease to occur, the infection must be created only by virulent strains. It is important to note that, loNDV does not produce ND. However, in the presence of different infections and inappropriate environmental conditions, these loNDV strains can also lead to some clinical respiratory problems (Diel et al., 2012; Dimitrov et al., 2016).

For effective disease management, it is crucial to be able to identify birds infected with NDV, as well as to distinguish vaccine viruses or loNDV strains and vNDV strains. Tracheal, oropharyngeal, and/or cloacal swabs from live animals are sent to the laboratory for organ identification from the dead animals. The diagnosis of ND can now be made by virus isolation and characterization methods such as Intracerebral Pathogenicity Index (ICPI). However, because these methods take a long time, nowadays, the commercial and "in-house" ND RT-PCR tests can differentially diagnose the meso/velogenic and lentogenic NDVs. F protein plays an essential role in the evaluation of virus virulence. In this context, the presence or absence of phenylalanine in *F* gene position 117, the number of basic amino acids encoded by this gene, is used as an essential marker of virulence. Different genotypes formed by the mutations in the genes encoding HN and F proteins may cause problems about the protection (Bello et al., 2018a).

Vaccination is the most effective method of prevention in the control of ND. Vaccination must be applied with strict biosafety measures. For this purpose, live vaccines and inactive vaccines are currently used

against ND. As live vaccines, there are lentogenic strains (Hitchner B1, Lasota, VG/GA), apathogenic enteric strains (Ulster 2C, V4), or mesogenic strains (Mukteswar). While live NDV vaccines can be administered collectively via drinking water, spray, or aerosols, inactivated vaccines are administered individually and therefore require more workforce than live vaccines. The correct particle size is essential for creating an appropriate immune response in spray or aerosol vaccination used for live vaccines. If the particles are too small, due to the accumulation of the virus in the lungs, the respiratory disease may occur, and vaccination reactions may develop, whereas if the particles are too large, the optimal immune response may not occur. Some live vaccine viruses with an ICPI value greater than or equal to 0.7 are also injected intradermally under the wing to reduce the severity of respiratory disease from the vaccine (Miller & Koch, 2020). Production of inactivated vaccines is more expensive and more difficult to administer than live vaccines. It is given intramuscularly or subcutaneously. Inactivated vaccines are the most suitable vaccines for immunity-boosting activity in animals and environmental safety (Miller & Koch, 2020; OIE World Organisation for Animal Health, 2018). On the other hand, inactivated vaccines lead to a limited time of immune response, they do not induce cell-mediated immunity, memory in immunity, and mucosal immunity, and thus, they are not considered to be very immunologically appropriate to protect chickens against NDV infection properly and for application as mass vaccination of the flocks by drinking water or spray ways. Apart from these live and inactivated vaccines, vector vaccines (recombinant vaccines) are also being used against ND. Vector vaccines are Fowl Pox or Marek Herpesvirus serotype 3 (Turkey Herpesvirus: HVT)-based vaccines that carry the fusion glycoprotein of NDV. These vaccines can be given to *in ovo* or to 0-day-old chicks in the hatchery. It does not produce vaccinal adverse reactions especially in the trachea of vaccinated chicks and can be applied in the presence of maternal immunity (Palya et al., 2012; Rauw et al., 2010).

Newcastle disease vaccines are prepared with genotype I (Ulster 2C and V4) and genotype II (LaSota, Hitchner B1, VG/GA, and Clone 30)-based strains. Apart from that, although only one mesogenic Mukteswar strain (genotype III) is used in live vaccine production, it always carries the risk of disease and forms very severe morbidity for chicks. Therefore, Mukteswar live vaccine is used only under certain conditions in chickens older than 4 weeks under control (Maminiaina et al., 2010; Miller et al., 2015).

Newcastle disease virus has one serotype (Bello et al., 2018a). Although this is regarded as an advantage for protection, ND cases are still reported all over the world today. This clearly shows that many issues need to be corrected regarding ND vaccines and vaccine effectiveness. Situations that may cause problems with ND vaccination and their possible solutions are summarized as follows:

1. Newcastle disease virus attenuated live vaccine strains are susceptible to heat. In this case, the cold chain in tropical and sub-tropical countries such as Turkey in terms of continuity can create problems. Therefore, providing immunization with more thermostable vaccine strains is seen as a more suitable method.
2. After the first day of vaccination of the 0-day-old chicks, the production of antibodies against NDV, which provides the protective immunity, starts at 6 days of age, and the protective antibody peak forms at approximately 21 days of age. Therefore, chicks are vulnerable to infection especially within these 6 days of life till vaccine-induced antibody formation. To shorten or minimize this open period, embryonic vaccinations can be applied in vivo with more attenuated (lentogenic) strains or vector ND vaccines. These practices play an essential role in providing early immunity.
3. The vaccine strains do not overlap antigenically with field strains. Since NDV is a single serotype, it is thought that protection against all virulent NDVs can be achieved with the vaccine. However, this does not mean that existing commercial vaccines completely prevent virus infection and that no virulent strains will be spilled from the vaccinated chickens. The protection that can be provided is related to the antigenic relatedness between the vaccine strain and virulent field strain. F protein is the most immunogenic antigen of NDV and is responsible for the production of neutralization antibodies. As a result, cross-protection between vaccine and field strain mainly depends on the *F* gene diversity between strains. Today, the *F* gene sequence difference among the 18 NDV genotypes identified in class II is between 8% and 29%. To date, all commercial live vaccines are produced from genotypes I and II isolated 70 years ago (Dimitrov et al., 2016; Maminiaina et al., 2010). *F* gene difference between current NDV field strains (genotypes I, VII, and XI–XVIII) and vaccine strains is higher than 16% (Dimitrov et al., 2016) (Table 2). In this case, current commercial live vaccines cannot provide adequate protection, even when vaccination is optimal. Today, we know that the F-genotype of NDV responsible for ND cases in backyard poultry and pigeons is NDV genotype VIIi and genotype IV, respectively, in the world (Fuller et al., 2017). Fuller et al. (2017) reported that the NDV VIIi was isolated from backyard chickens in Turkey. Moreover, Turan et al. (2020) have demonstrated the presence of subgenotype VII.2 of NDV in wild birds. On the other hand, if a large-scale epidemiological study covering all backyard, domestic, and wild birds in Turkey is carried out, it may be possible to detect different types of NDV genotypes in different geographical regions of Turkey. Therefore, we strongly advise to Turkish poultry sector to conduct an epidemiological study which will show the profile of NDV genotypes responsible for NDV infections in poultry flocks even if they were vaccinated with classical vaccine strains belonging to genotype II.

A strong immunity on a herd basis in ND vaccination can be achieved by the fact that the antibody titer of a large part of the flock (>85%) is greater than $\log_2 3$ after inoculation. If the average antibody titer acquired by vaccination is below the $\log_2 3$ and the virulent field viruses are different from the current vaccine strain, field virus find also a chance to infect birds and to spread to other birds in the flocks, finally resulting in ND cases (van Boven et al., 2008). Therefore, antigenically matched and quality live vaccines and vaccination strategies may be critical in suppressing viral shedding of existing field viruses and, ultimately, in the control of ND outbreaks. Therefore, from the logical perspective, we really urgently need a molecular epidemiological study showing the up-to-date distribution of NDV genotypes responsible for ND cases in the regions or companies in Turkey, and some genetically engineered live vaccines containing an NDV genotype that matches the NDV genotypes in the field can be tried as an alternative.

Avian Influenza Virus Infection

Avian influenza virus (AIV) infection which is the third pandemic infection among avian species causes two types of disease (World

Health Organization [WHO], 2005). The first one is the high-pathogenic peracute or acute progressive Fowl Plague Disease observed with approximately 100% mortality caused by highly pathogenic AIV (HPAIV), the second is a respiratory infection caused by AIVs with low pathogenicity (low-pathogenic AIV (LPAIV)), showing low mortality and causing significant low egg yields in laying hens as well as the respiratory tract infections (Swayne et al., 2020). Low-pathogenic AIV infections can also lead to 40–97% mortality in broilers. In turkeys, sinusitis and yield losses can reach significant levels. AI is among the notifiable diseases in our country. Avian influenza viruses are influenza A viruses, one of three genera (influenza A, B, C, and D) found in the *Orthomyxoviridae* family. These viruses are the agents that contain negative-sense, segmented, single-stranded RNA (Kim et al., 2016). Avian influenza virus genome encodes eight structural proteins including hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix 1 (M1), matrix 2 (M2), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA) (Swayne et al., 2020). Avian influenza viruses s have subtypes according to the type of HA and N proteins. Accordingly, AIVs have 16 HA types and 9 N types. Considered in this way, so far, the plague agents (HPAIV) in poultry are H5 and H7 types. However, not all of these H5 and H7 types cause HPAIV disease, so H5 and H7 can also have low pathogenic types. Table 3 represents the H5 and H7 subtypes in chicken. The H5 and H7 HPAIVs can also cause lethal disease in humans, and (Lee et al., 2020; Sutton, 2018; Tang et al., 2021) they are considered as important zoonotic viruses, while LPAIVs of other HA types, despite being zoonotic, have not been reported to cause disease in humans although they are capable of causing infections.

In poultry flocks, LPAIVs can create infections alone, as well as multiple infections in the form of dual, triple, and even quadruple different LPAIVs. In this context, it should be kept in mind that more than one LPAIV serotype can be found in the same sample in virus screening in our flocks. However, it is possible to observe LPAIV infections in the field, together with bacterial infections such as *Escherichia coli, Pasteurella* spp, pathogenic *Mycoplasma* infections, and viral infections such as ND, IB infections (Roussan et al., 2008; Samy & Naguib, 2018; Sid et al., 2015). In this context, considering the multifactorial infections is a very critical situation in terms of designing protection control strategies. A high number of AIV variants, the presence of other pathogenic infectious agents, the amount of dust and ammonia in the poultry house, and heat stress increase infection severity and economical losses (Yu et al., 2015).

Table 3

Representative Strains of H5 and H7 Subtypes of Avian Influenza Virus in Chicken

Subtype	Strain	Country	Date
H5N1	A/chicken/France/150169a/2015	France	2015
H5N2	A/chicken/Changhua/15010120-1/2015	Taiwan	2015
H5N3	A/chicken/Taiwan/01174/2015	Taiwan	2015
H5N4	A/chicken/Iraq/KCVL-VI/2015	Iraq	2015
H5N6	A/chicken/HeBei/CK05/2019	China	2019
H5N8	A/chicken/Pakistan/531/2018	Pakistan	2018
H5N9	A/chicken/Italy/9097/1997	Italy	1997
H7N1	A/chicken/Rostock/R0p/1934	Germany	1934
H7N2	A/chicken/NJ/294598-MA/2004	USA	2004
H7N3	A/chicken/BC/CN-006/2004	Canada	2004
H7N4	A/chicken/Jiangsu/1/2018	China	2018
H7N6	A/chicken/Zhejiang/233/2016	China	2016
H7N7	A/chicken/Cambodia/Z437W28M3/2015	Cambodia	2015

Diagnosis of LPAIV and HPAIV infections is done by virus isolation and/or detecting virus RNA from clinical specimens (trachea, tracheal swab, stool, and other organ samples with lesions) and type determination of the isolated virus (OIE World Organisation for Animal Health, 2018). Virus isolation and characterization must be done in laboratories with Biosafety Level (BSL) 3 standard with BSL 2 workflow. The most commonly used molecular technique is RT-PCR that enables the rapid detection and subtyping of causative agents in clinical specimens. Also, qRT-PCR is frequently used for detecting influenza A viral genomes and for virus subtyping (Okamatsu et al., 2016). The rapid isothermal amplification technologies, such as loop-mediated isothermal amplification (LAMP) method, have been used to detect H5N1, H5N6, H5N8, and H7N9 within an hour (Ahn et al., 2019). Nucleic acid sequence-based amplification assays have been developed for the detection of AI virus subtypes including H7 and H5 in clinical samples within 6 hours (Lau et al., 2004).

Briefly, to isolate the virus from the clinical samples, inoculation of specific pathogen-free embryonated chicken eggs or specific antibody-negative eggs is routinely done (Spackman & Killian, 2014). The presence of the virus in the allantoic fluid of the inoculated embryonated eggs is detected by using hemagglutination inhibition (HI) test and specific RT-PCR methods. The presence of the virus from the clinical samples can also be directly shown by molecular techniques such as RT-PCR or LAMP (Ahn et al., 2019; Bao et al., 2014). After detecting or isolating the AIV from the samples, AIVs should be subtyped according to their HA and N protein differences. For this, HA, HI, and RT-PCR tests were practically used in the reference laboratories (Ahn et al., 2019; James et al., 2019; Kwon et al., 2019). Epidemiologically, to monitor the AIV, sequencing of HA and N genes of the isolated or detected viruses is the most promising method to evaluate antigentic drifts and shifts that occurred in the viruses (Rauw et al., 2011; Swayne et al., 2015). Genotyping of the AIV by sequencing is also informative to see clonal relations and to follow the clonal or different virus circulations in poultry and the migratory birds, which are carriers of the AIV without showing any symptoms. In addition to this, genetic information of the AIV is also useful for determining the resistance of the virus to the anti-viral agents. This is very important to human medicine to treat the human AI cases because AIVs are potentially one of the most important zoonotic agents (Cox et al., 2016).

Vaccination has always been a critical control option in regions where LPAIV infections are a frequent problem (Food and Agriculture Organization of the United Nations [FAO], 2016; Lee et al., 2016). Of course, identification of the HA serotypes circulating in the field before vaccination is essential for determining the protectotype vaccine (Ge et al., 2007). For example, in the United States and Mexico, during outbreaks of LPAIV H9N2 and H5N2, respectively, vaccines were used effectively in the role of infection control. In Italy, vaccines have been successfully administered in

turkeys in H6N2 and H9N2 outbreaks (Alexander, 2007). In order to activate the vaccination option, considering the size and extent of the disease problem has a critical value. However, it should be kept in mind that if the marker vaccine is not applied, it will be very difficult to distinguish between infected and vaccinated animals after vaccination. As in the case of NDV, the poultry sector itself should also continuously monitor the LPAIV HA types circulating in Turkish poultry flocks by genetics and virological techniques, although responsible reference laboratories belonging to the Ministry of Agriculture and Forestry of Turkey have been examining two times (in the autumn and spring sessions of each year) a year the AIV HA types and their antibodies from samples from commercial and backyard poultries and aquatic birds to declare the AI situation of Turkey to OIE for a long time. Vaccines containing antigenically or genetically matched viruses to the field viruses to be possibly circulated in Turkey poultry flocks are held as an option to control the infection when a contingency plan for AI is implemented to the country by the Ministry. In the case of implementation of the vaccination strategy as an option in a country to reduce the level of AIV infections in the poultry flocks and for surveillance of the infection the wild birds including migratory birds, serological tests such as HI and ELISA are widely used to detect the antibodies against the AIV HA serotypes (Arnold et al., 2018). Neurominidase inhibition test is another serological method to be used for differentiating the infected from vaccinated animals which is a strategy used in the reference laboratories (Avellaneda et al., 2010).

Conclusion and Recommendations

The economic effects of respiratory virus infections are immense in poultry companies. The implementation of correct prevention programs and vaccination strategies depends on the correct molecular detection and genetic and antigenic characterization of infectious agents, as well as the coexistence of these infectious viruses and their genotypes in the host. Because of this, historical progress in the genetic changes and control strategies used against these avian pandemic viruses by using molecular methods will elucidate the way of control and vaccine strategies.

In this context, considering the multifactorial etiology in the diagnosis of respiratory virus infections, the selection of suitable modern genetic and molecular rapid diagnosis techniques in determining the genotypes and serotypes of the viral agents is of great importance. As a result, a valid and current prevention and control strategy can be designed by the correct updated molecular characterization of pathogenic viruses and bacteria, selection of appropriate diagnostic methods including culture, PCR, qPCR, or other nucleic acid amplification methods such as LAMP or circular amplification technology, sequencing, and evaluation of reliable results to be obtained within the scope of combating avian respiratory virus diseases.

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References

Afonso, C. L., Amarasinghe, G. K., Bányai, K., Báo, Y., Basler, C. F., Bavari, S., Bejerman, N., Blasdell, K. R., Briand, F. X., Briese, T., Bukreyev, A., Calisher, C. H., Chandran, K., Chéng, J., Clawson, A. N., Collins, P. L., Dietzgen, R. G., Dolnik, O., Domier, L. L., Dürrwald, R., et al. (2016). Taxonomy of the order Mononegavirales: update 2016. *Archives of Virology*, *161*, 2351–2360.

Ahn, S. J., Baek, Y. H., Lloren, K. K. S., Choi, W. S., Jeong, J. H., Antigua, K. J. C., Kwon, H. I., Park, S. J., Kim, E. H., Kim, Y. I., Si, Y. J., Hong, S. B., Shin, K. S., Chun, S., Choi, Y. K., & Song, M. S. (2019). Rapid and simple colorimetric detection of multiple influenza viruses infecting humans using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform. *BMC Infectious Diseases*, *19*(1), 676. [CrossRef]

Alexander, D. J. (2007). An overview of the epidemiology of avian influenza. *Vaccine*, *25*(30), 5637–5644. [CrossRef]

Arnold, M. E., Slomka, M. J., Breed, A. C., Hjulsager, C. K., Pritz-Verschuren, S., Venema-Kemper, S., Bouwstra, R. J., Trebbien, R., Zohari, S., Ceeraz, V., Larsen, L. E., Manvell, R. J., Koch, G., & Brown, I. H. (2018). Evaluation of ELISA and haemagglutination inhibition as screening tests in serosurveillance for H5/H7 avian influenza in commercial chicken flocks. *Epidemiology and Infection*, *146*(3), 306–313. [CrossRef]

Avellaneda, G., Mundt, E., Lee, C. W., Jadhao, S., & Suarez, D. L. (2010). Differentiation of infected and vaccinated animals (DIVA) using the NS1 protein of avian influenza virus. *Avian Diseases*, *54*(Suppl. 1), 278–286. [CrossRef]

Bande, F., Arshad, S. S., Hair-Bejo, M., Moeini, H., & Omar, A. R. (2015). Progress and challenges toward the development of vaccines against avian infectious bronchitis. *Journal of Immunology Research*, *2015*, 424860. [CrossRef]

Bao, H., Zhao, Y., Wang, Y., Xu, X., Shi, J., Zeng, X., Wang, X., & Chen, H. (2014). Development of a reverse transcription loop-mediated isothermal amplification method for the rapid detection of subtype H7N9 avian influenza virus. *BioMed Research International*, *2014*, 525064. [CrossRef]

Bello, M. B., Yusoff, K., Ideris, A., Hair-Bejo, M., Peeters, B. P. H., & Omar, A. R. (2018a). Diagnostic and vaccination approaches for Newcastle disease virus in poultry: The current and emerging perspectives. *BioMed Research International*, *2018*, 7278459. [CrossRef]

Bello, M. B., Yusoff, K. M., Ideris, A., Hair-Bejo, M., Peeters, B. P. H., Jibril, A. H., Tambuwal, F. M., & Omar, A. R. (2018b). Genotype diversity of Newcastle disease virus in Nigeria: Disease control challenges and future outlook. *Advances in Virology*, *2018*, 6097291. [CrossRef]

Blakey, J., Crossley, B., Da Silva, A., Rejmanek, D., Jerry, C., Gallardo, R. A., & Stoute, S. (2020). Infectious bronchitis virus associated with nephropathy lesions in diagnostic cases from commercial broiler chickens in California. *Avian Diseases*, *64*(4), 482–489. [CrossRef]

Carlı, K. T. (2019). *Kanatlı hayvanlarin enfeksiyon hastaliklari*. Ankara: Nobel Tıp Press.

Chacón, R. D., Astolfi-Ferreira, C. S., Chacón, J. L., Nuñez, L. F. N., De la Torre, D. I., & Piantino Ferreira, A. J. (2019). A seminested RT-PCR for molecular genotyping of the Brazilian BR-I Infectious Bronchitis Virus Strain (GI-11). *Molecular and Cellular Probes*, *47*, 101426. [CrossRef]

Cox, N. J., Trock, S. C., & Uyeki, T. M. (2016). Public health implications of animal influenza viruses. *Animal Influenza*, 92–132.

de Almeida, R. S., Hammoumi, S., Gil, P., Briand, F. X., Molia, S., Gaidet, N., Cappelle, J., Chevalier, V., Balança, G., Traoré, A., Grillet, C., Maminiaina, O. F., Guendouz, S., Dakouo, M., Samaké, K., Bezeid, Oel M., Diarra, A., Chaka, H., Goutard, F., Thompson, P., et al. (2013). New avian paramyxoviruses type I strains identified in Africa provide new outcomes for phylogeny reconstruction and genotype classification. *PLoS One*, *8*(10), e76413. [CrossRef]

de Wit, J. J., Dijkman, R., Guerrero, P., Calvo, J., Gonzalez, A., & Hidalgo, H. (2017). Variability in biological behaviour, pathogenicity, protectotype and induction of virus neutralizing antibodies by different vaccination

programmes to infectious bronchitis virus genotype Q1 strains from Chile. *Avian Pathology*, *46*(6), 666–675. [CrossRef]

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. [CrossRef]

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. [CrossRef]

Food and Agriculture Organization of the United Nations (FAO). (2016). *Rational use of vaccination for prevention and control of H5 highly pathogenic avian influenza. Focus on*, *10* (pp. 1–12). Rome, Italy: Food and Agriculture Organization.

Fuller, C., Löndt, B., Dimitrov, K. M., Lewis, N., van Boheemen, S., Fouchier, R., Coven, F., Goujgoulova, G., Haddas, R., & Brown, I. (2017). An epizootiological report of the re-emergence and spread of a lineage of virulent Newcastle disease virus into Eastern Europe. *Transboundary and Emerging Diseases*, *64*(3), 1001–1007. [CrossRef]

Ge, J., Deng, G., Wen, Z., Tian, G., Wang, Y., Shi, J., Wang, X., Li, Y., Hu, S., Jiang, Y., Yang, C., Yu, K., Bu, Z., & Chen, H. (2007). Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses. *Journal of Virology*, *81*(1), 150–158. [CrossRef]

Han, Z., Liwen, X., Ren, M., Sheng, J., Ma, T., Sun, J., Zhao, Y., & Liu, S. (2020). Genetic, antigenic and pathogenic characterization of avian coronaviruses isolated from pheasants (Phasianus colchicus) in China. *Veterinary Microbiology*, *240*, 108513. [CrossRef]

Jackwood, M. W., & de Witt, S. (2020). Infectious bronchitis. In D. E. Swayne, et al. (Eds.), *Diseases of poultry* (pp. 167–188). Wiley-Blackwell.

Jackwood, M. W., Hall, D., & Handel, A. (2012). Molecular evolution and emergence of avian gammacoronaviruses. *Infection, Genetics and Evolution*, *12*(6), 1305–1311. [CrossRef]

James, J., Slomka, M. J., Reid, S. M., Thomas, S. S., Mahmood, S., Byrne, A. M. P., Cooper, J., Russell, C., Mollett, B. C., Agyeman-Dua, E., Essen, S., Brown, I. H., & Brookes, S. M. (2019). Proceedings paper: Avian diseases 10th AI symposium issue development and application of real-time PCR assays for specific detection of contemporary avian influenza virus Subtypes N5, N6, N7, N8, and N9. *Avian Diseases*, *63*(sp1), 209–218. [CrossRef]

Ji, J., Xie, J., Chen, F., Shu, D., Zuo, K., Xue, C., Qin, J., Li, H., Bi, Y., Ma, J., & Xie, Q. (2011). Phylogenetic distribution and predominant genotype of the avian infectious bronchitis virus in China during 2008–2009. *Virology Journal*, *8*(1), 184. [CrossRef]

Jordan, B. (2017). Vaccination against infectious bronchitis virus: A continuous challenge. *Veterinary Microbiology*, *206*, 137–143. [CrossRef]

Kahya, K., Coven, F., Temelli, S., Eyigor, A., & Carli, K. T. (2013). Presence of IS/1494/06 genotype-related infectious bronchitis virus in breeder and broiler flocks in Turkey. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *60*(1), 27–31. [CrossRef]

Khataby, K., Kasmi, Y., Souiri, A., Loutfi, C., & Ennaji, M. M. (2020). Avian coronavirus: Case of infectious bronchitis virus pathogenesis, diagnostic approaches, and phylogenetic relationship among emerging strains in Middle East and North Africa regions. *Emerging and Reemerging Viral Pathogens*, *2020*, 729–744.

Kim, S. M., Kim, Y. I., Pascua, P. N. Q., & Choi, Y. K. (2016). Avian influenza A viruses: Evolution and zoonotic infection. *Seminars in Respiratory and Critical Care Medicine*, *37*(4), 501–511. [CrossRef]

Kwon, N., Ahn, J. J., Kim, J. H., Kim, S., Lee, J. H., Kwon, J. H., Song, C. S., & Hwang, S. Y. (2019). Rapid subtyping and pathotyping of avian influenza virus using Chip-based RT-PCR. *BioChip Journal*, *13*(4), 333–340.

Lau, L. T., Banks, J., Aherne, R., Brown, I. H., Dillon, N., Collins, R. A., Chan, K. Y., Fung, Y. W. W., Xing, J., & Yu, A. C. (2004). Nucleic acid sequence-based amplification methods to detect avian influenza virus. *Biochemical and Biophysical Research Communications*, *313*(2), 336–342. [CrossRef]

Lee, D. H., Fusaro, A., Song, C. S., Suarez, D. L., & Swayne, D. E. (2016). Poultry vaccination directed evolution of H9N2 low pathogenicity avian influenza viruses in Korea. *Virology*, *488*, 225–231. [CrossRef]

Lee, Y. N., Lee, D. H., Cheon, S. H., Park, Y. R., Baek, Y. G., Si, Y. J., Kye, S. J., Lee, E. K., Heo, G. B., Bae, Y. C., Lee, M. H., & Lee, Y. J. (2020). Genetic characteristics and pathogenesis of H5 low pathogenic avian influenza viruses from wild birds and domestic ducks in South Korea. *Scientific Reports*, *10*(1), 12151. [CrossRef]

Li, L., Xue, C., Chen, F., Qin, J., Xie, Q., Bi, Y., & Cao, Y. (2010). Isolation and genetic analysis revealed no predominant new strains of avian infectious bronchitis virus circulating in South China during 2004–2008. *Veterinary Microbiology*, *143*(2–4), 145–154. [CrossRef]

Lin, S. Y., & Chen, H. W. (2017). Infectious bronchitis virus variants: Molecular analysis and pathogenicity investigation. *International Journal of Molecular Sciences*, *18*(10), 2030. [CrossRef]

Maminiaina, O. F., Gil, P., Briand, F. X., Albina, E., Keita, D., Andriamanivo, H. R., Chevalier, V., Lancelot, R., Martinez, D., Rakotondravao, R., Rajaonarison, J. J., Koko, M., Andriantsimahavandy, A. A., Jestin, V., & Servan de Almeida, R. S. (2010). Newcastle disease virus in Madagascar: Identification of an original genotype possibly deriving from a died out ancestor of genotype IV. *PLOS ONE*, *5*(11), e13987. [CrossRef]

Miller, P. J., & Koch, G. (2020). Newcastle disease. In D. E. Swayne, et al. (Eds.), *Diseases of poultry* (pp. 112–129). Wiley-Blackwell.

Miller, P. J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S. F., Wajid, A., Bibi, T., Khan, T. A., Yaqub, T., Setiyaningsih,S., & Afonso, C. L. (2015). Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infection, Genetics and Evolution* , *29*, 216–229. [CrossRef]

Montassier, H. J. (2010). Molecular epidemiology and evolution of avian infectious bronchitis virus. *Revista Brasileira de Ciência Avícola*, *12*(2), 87–96. [CrossRef]

Najafi, H., Langeroudi, A. G., Hashemzadeh, M., Karimi, V., Madadgar, O., Ghafouri, S. A., Maghsoudlo, H., & Farahani, R. K. (2016). Molecular characterization of infectious bronchitis viruses isolated from broiler chicken farms in Iran, 2014–2015. *Archives of Virology*, *161*(1), 53–62. [CrossRef]

OIE World Organisation for Animal Health (2018). Manual of diagnostic tests and vaccines for terrestrial animals [Online]. Retrieved from https://www.oie.int/standard-setting/terrestrial-manual/access-online/.

Okamatsu, M., Hiono, T., Kida, H., & Sakoda, Y. (2016). Recent developments in the diagnosis of avian influenza. *Veterinary Journal*, *215*, 82–86. [CrossRef]

Palya, V., Kiss, I., Tatár-Kis, T., Mató, T., Felföldi, B., & Gardin, Y. (2012). Advancement in vaccination against Newcastle disease: Recombinant HVT NDV provides high clinical protection and reduces challenge virus shedding with the absence of vaccine reactions. *Avian Diseases*, *56*(2), 282–287. [CrossRef]

Rauw, F., Gardin, Y., Palya, V., Anbari, S., Lemaire, S., Boschmans, M., van den Berg, T., & Lambrecht, B. (2010). Improved vaccination against Newcastle disease by an in ovo recombinant HVT-ND combined with an adjuvanted live vaccine at day-old. *Vaccine*, *28*(3), 823–833. [CrossRef]

Rauw, F., Palya, V., Van Borm, S., Welby, S., Tatar-Kis, T., Gardin, Y., Dorsey, K. M., Aly, M. M., Hassan, M. K., Soliman, M. A., Lambrecht, B., & Van den Berg, T. (2011). Further evidence of antigenic drift and protective efficacy afforded by a recombinant HVT-H5 vaccine against challenge with two antigenically divergent Egyptian clade 2.2. 1 HPAI H5N1 strains. *Vaccine*, *29*(14), 2590–2600. [CrossRef]

Ren, G., Liu, F., Huang, M., Li, L., Shang, H., Liang, M., Luo, Q., & Chen, R. (2020). Pathogenicity of a QX-like avian infectious bronchitis virus isolated in China. *Poultry Science*, *99*(1), 111–118. [CrossRef]

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

Rui, Z., Juan, P., Jingliang, S., Jixun, Z., Xiaoting, W., Shouping, Z., Xiaojiao, L., & Guozhong, Z. (2010). Phylogenetic characterization of Newcastle disease virus isolated in the mainland of China during 2001–2009. *Veterinary Microbiology*, *141*(3–4), 246–257. [CrossRef]

Samy, A., & Naguib, M. M. (2018). Avian respiratory coinfection and impact on avian influenza pathogenicity in domestic poultry: Field and experimental findings. *Veterinary Sciences*, *5*(1), 23. [CrossRef]

Schalk, A. (1931). An apparently new respiratory disease of baby chicks. *Journal of the American Veterinary Medical Association*, *78*, 413–423.

Sid, H., Benachour, K., & Rautenschlein, S. (2015). Co-infection with multiple respiratory pathogens contributes to increased mortality rates in Algerian poultry flocks. *Avian Diseases*, *59*(3), 440–446. [CrossRef]

Smialek, M., Tykalowski, B., Dziewulska, D., Stenzel, T., & Koncicki, A. (2017). Immunological aspects of the efficiency of protectotype vaccination strategy against chicken infectious bronchitis. *BMC Veterinary Research*, *13*(1), 44. [CrossRef]

Spackman, E., & Killian, M. L. (2014). *Avian influenza virus isolation, propagation, and titration in embryonated chicken eggs. Animal influenza virus* (pp. 125–140). New York, NY: Humana Press.

Sutton, T. C. (2018). The pandemic threat of emerging H5 and H7 avian influenza viruses. *Viruses*, *10*(9), 461. [CrossRef]

Swayne, D. E., Suarez, D. L., & Sims, L. D. (2020). Influenza. In D. E. Swayne, et al. (Eds.), *Diseases of poultry* (pp. 210–256). Wiley-Blackwell.

Swayne, D. E., Suarez, D. L., Spackman, E., Jadhao, S., Dauphin, G., Kim-Torchetti, M., McGrane, J., Weaver, J., Daniels, P., Wong, F., Selleck, P., Wiyono, A., Indriani, R., Yupiana, Y., Sawitri Siregar, E., Prajitno, T., Smith, D., & Fouchier, R. (2015). Antibody titer has positive predictive value for vaccine protection against challenge with natural antigenic-drift variants of H5N1 high-pathogenicity avian influenza viruses from Indonesia. *Journal of Virology*, *89*(7), 3746–3762. [CrossRef]

Tang, W., Li, X., Tang, L., Wang, T., & He, G. (2021). Characterization of the low-pathogenic H7N7 avian influenza virus in Shanghai, China. *Poultry Science*, *100*(2), 565–574. [CrossRef]

Turan, N., Ozsemir, C., Yilmaz, A., Cizmecigil, U. Y., Aydin, O., Bamac, O. E., Gurel, A., Kutukcu, A., Ozsemir, K., Tali, H. E., Tali, B. H., Yilmaz, S. G., Yaramanoglu, M., Tekelioğlu, B. K., Ozsoy, S., Richt, J. A., Iqbal, M., & Yilmaz, H. (2020). Identification of Newcastle disease virus subgenotype VII. 2 in wild birds in Turkey. *BMC Veterinary Research*, *16*(1), 277. [CrossRef]

Valastro, V., Holmes, E. C., Britton, P., Fusaro, A., Jackwood, M. W., Cattoli, G., & Monne, I. (2016). S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. *Infection, Genetics and Evolution*, *39*, 349–364. [CrossRef]

van Boven, M., Bouma, A., Fabri, T. H., Katsma, E., Hartog, L., & Koch, G. (2008). Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian Pathology* , *37*(1), 1–5. [CrossRef]

Welch, B. D., Yuan, P., Bose, S., Kors, C. A., Lamb, R. A., & Jardetzky, T. S. (2013). Structure of the parainfluenza virus 5 (PIV5) hemagglutinin-neuraminidase (HN) ectodomain. *PLoS Pathogens*, *9*(8), e1003534. [CrossRef].

World Health Organization (2005). *Avian influenza: Assessing the pandemic threat* (No. WHO/CDS/2005.29). Retrieved from https://apps.who.int/iris/handle/10665/68985.

Yu, J., Shi, F. S., & Hu, S. (2015). Improved immune responses to a bivalent vaccine of Newcastle disease and avian influenza in chickens by ginseng stem-leaf saponins. *Veterinary Immunology and Immunopathology*, *167*(3–4), 147–155. [CrossRef]