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Study of the prevalence of viral respiratory diseases in poultry in Dakahlia Governorate during 2017-2018

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Abstract

In this work, respiratory viral diseases (AI, IBV and NDV) were detected using real-time RT-PCR in 30 broiler flocks suffering from respiratory signs in Dakahlia province between January 2017 and August 2018. Mixed infectious bronchitis (IBV) and avian influenza (AI-H9N2) viruses were the most dominant infection (40%). The high incidence of mixed illnesses including AI-H9N2 and the absence of AI-H9N2 immunization point to an early AI-H9N2 infection that may have immunosuppressive effects and predispose to other viral diseases. Moreover, 10% of cases of virulent Newcastle disease virus (vNDV) infections were found. Using the hemagglutinin gene (HA), fusion gene, and S1 gene hypervariable region 3 (HVR3) sequences, very frequent IBV, AI-H9N2, and NDV were identified and molecularly described. IBV strains with several HVR3 mutations were connected to the variant II group of IBV. Even though AI-H9N2 viruses evolved more slowly than more recent strains, the HA gene showed a small number of amino acid changes that suggested antibodies selection pressure. Additionally, NDV genotype VII was found and its fusion gene sequenced. All samples were tested negative for AIH5. In conclusion, the most common viruses found in broiler chickens in Dakahlia during the study period were mixed viral pathogens, particularly those including IBV and AI-H9N2 viruses.

Keywords: *Poultry, Infectious bronchitis virus, Newcastle disease virus, co-infection, avian influenza virus.*

Introduction

Due to their complex character, respiratory illnesses provide a significant challenge to the chicken business (Roussan et al., 2008). A number of avian viruses preferentially target the hen's respiratory system. The respiratory tract of hens with high mortality is largely infected by infectious laryngeotrachitis virus (ILT), Newcastle disease virus (NDV), avian influenza virus (AIV), infectious bronchitis virus (IBV), and pneumovirus (Haghighat-Jahromi et al., 2008). Live and inactivated vaccinations have been developed and used for many years by the chicken industry to control most respiratory infections. According to Parsons et al. (1992), the condition in the field was not under control with the commercially available vaccinations. Lack of vaccination coverage, mismatched vaccine strains, or delayed biosecurity are the main causes of Egypt's inability to control poultry diseases. Numerous nations, including Egypt, have seen an endemic spread of AI-H5N1 viruses in poultry as a result of extensive surveillance and genetic research. Avian influenza epidemiology in Egypt is thought to be significantly influenced by clinically undetectable, free-ranging infected ducks and geese, as well as mixed species backyard holdings (Aly et al., 2008). An additional difficulty facing Egypt's poultry business was the discovery of the AI-H9N2 subtype in 2010 and 2011 in commercial quail and chickens (El-Zoghby et al., 2012). Egypt is endemic for both H5N1 and H9N2 viruses, molecular characterization of AIVs is important for

screening the molecular evolution of each subtype and potential recombination events.

Even with routine immunizations against strains of the virus from Massachusetts (Mass), IBV continues to have a variety of consequences on Egypt's chicken sector. Since the isolation of IB variant that neutralization testing revealed to be closely linked to the Dutch variant D3128 IBV has been recognized (Sheble et al., 1986). Subsequently, variants related to Mass (Eid, 1998), D3128, D274, D-08880, and 4/91 were characterized. Novel isolates Egypt/Beni-Suef/01 and Egypt/Beni-Suef/03, nephropathogenic IBV strains closely linked to Israeli variant 2 and Mass serotype, respectively, revealed novel genotypes endemic in Egypt (Abdel-Moneim et al., 2006). Other unique genotypes have been identified as Egyptian variant I (e.g., CK/Eg/BSU-1,4,5/2011) and Egyptian variant II (e.g., CK/Eg/BSU-2,3/2011) that were reported in Egypt recently.

Despite rigorous immunization programs, the NDV has posed a severe threat to chicken production in many nations, despite massive attempts to contain the illness. It was determined that NDV strains from recent outbreaks that struck poultry farms in Egypt in 2011 and 2012 belonged to class II, genotype VII, sub genotype d. According to Mohammed et al. (2011) and Radwan et al. (2013), trade in chicken and poultry products with China and other Middle Eastern nations is the likely source of the spread of genotype VII in Egypt.

Although it can be expensive, labor-intensive, and sluggish, virus isolation is the standard technique for diagnosing avian respiratory viruses (Suarez et al., 2007). Reverse transcriptase polymerase chain reaction (RT-PCR), real-time RT-PCR (rRT-PCR) and rapid chromatographic assays for antigen detection are among the quick diagnostic tools that have been developed for the detection of either viral nucleic acids or antigens (Spackman et al., 2002).

This study examined the presence of three viruses (AI, vNDV, and IBV) in chicken exhibiting respiratory symptoms in the province of Dakahlia using the rRT-PCR technology. Additionally, certain respiratory viruses underwent molecular analysis in order to investigate the genetic characteristics of the circulating viruses.

Materials and Methods

Field Samples

Tracheal or oropharyngeal swabs and tissues were collected from 50 broiler chicken farm (10 samples per flock) suffering from respiratory distress between January 2017 and August 2018 from different parts in dakahlia province. Tissue samples were ground in PBS pH 7.0 to 7.4 containing gentamycin (50 µg/mL) and mycostatin (1,000 units/mL) in a 1:5 (w/v) dilution, centrifuged, and tissue supernatant was used (OIE, 2014).

RNA Extraction and Real-Time RT-PCR (rRT-PCR)

Following the manufacturer's instructions, the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used to extract the viral RNA. Real-time reverse transcription polymerase chain reaction (rRT-PCR) was used to confirm the virus identification. Quantitect probe RT-PCR kit (Qiagen, Hilden, Germany) was utilized, and particular oligonucleotide primers and probes were used for each target virus. Regarding vNDV (Wise et al., 2004), IBV (Callison et al., 2006), H5N1 (Slomka et al., 2007), and AIH9N2 (Ben Shabat et al., 2010).

Virus Isolation

Based on rRT-PCR detection, seasonal, and fatality rates noted, eight samples were chosen for the very prevalent respiratory viruses (3 samples for IBV, 3 for NDV, and 2 AI-H9N2) (Table 1). After infected birds' trachea swabs and/or tissue suspensions were obtained, 10-day-old specific pathogen-free embryonated chicken eggs (SPF-ECE) were prepared for allantoic inoculation (OIE, 2014). For 96 hours, inoculated eggs were incubated at 37ºC, and their embryo viability was monitored every day. For isolation of IBV-positive rRT-PCR samples, three blind passages in SPF-ECE were carried out. 1% cleaned chicken red blood cells were used to evaluate all injected egg allantoic fluids from dead and surviving embryos for hemagglutination (OIE, 2014).

Gene sequencing and sequence analysis

The viral RNA was eluted from collected allantoic fluids by Qiagen one-step RT-PCR (Qiagen, Hilden, Germany), based on the man-

ufacturer's instructions. Using particular oligonucleotide primers for partial amplification of target genes of AI viruses (complete hemagglutinin gene (HA) gene; Shany, 2015), IBV (S1 gene); Selim et al., 2013); and vNDV genes (Wise et al., 2004), RT-PCR is employed for molecular characterizations of isolates. Using the Clustal W alignment tool, the MEGA version 6 program was used to identify sequence comparisons and phylogenetic relationships by a bootstrap of 1,000 trials (Tamura et al., 2013). Using the CLUSTAL W Multiple Sequence Alignment Program, version 1.83 of the MegAlign module of Lasergene DNAStar software, a comparative study of inferred amino acid and nucleotide sequences was produced (Ziegler et al., 2002). The GenBank database received sequences produced throughout the course of this investigation, with accession numbers displayed in Table 1.

Table (1). History of isolated viruses in the present study

Isolate	Virus	Date of collection	Age of birds/d	Mortality rate	Vaccination	GeneBank accession number
IBV-Ch/Egy-Dakahlia-1-2017-SP1	IBV	2017	35	3%	IB Hitchner (D7), IB-Clone (D15)	In Progress
IBV - Ch/Egy - Dakahlia -2-2017-SP1	IBV	2017	24	10%	IB Hitchner (D7) H5N1 (D9) IB-Clone (D15)	In Progress
IBV - Ch/Egy - Dakahlia -3-2018-SP1	IBV	2018	30	5%	IB (Ma 5) (D1), IB Hitchner (D7), IB-Clone (D15)	In Progress
NDV- Ch/Egy - Dakahlia -1-2017-Fu	NDV	2017	32	15%	IB Hitchner (D1), IB-Clone (D15)	In Progress
NDV- Ch/Egy - Dakahlia -2-2018-Fu	NDV	2018	29	10%	IB Hitchner (D7), H5N1 (D9) IB-Clone (D15)	In Progress
NDV- Ch/Egy - Dakahlia -3-2018-Fu	NDV	2018	36	20%	IB Hitchner (D7), IB-Clone (D15)	In Progress
A-Chicken- Dakahlia - 1-2017-H9N2	H9N2	2017	25	15%	IB Hitchner (D7), IB-Clone (D15)	In Progress
A-Chicken- Dakahlia -2-2018-H9N2	H9N2	2018	36	20%	IB Hitchner (D7), NDV Avinew (D17)	In Progress

Results

Clinical reports of examined farms

Examined were thirty broiler chicken flocks from the Dakahlia Governorate that had respiratory diseases. The respiratory disease outbreaks under investigation had diverse clinical presentations and postmortem lesions, contingent on the virus strain causing the illness, the flock's vaccination regimen, and the presence of multiple illnesses. The primary clinical signs included nasal discharges, rales, and respiratory distress manifested as gasping. Head tilting was noted as a neurotic symptom in certain flocks. Upon postmortem examination, the primary clinical characteristics observed were tracheitis, congested viscera, and tracheal caseation at the tracheal bifurcation.

Detection by rRT- PCR

The most frequent condition in the flocks under investigation was mixed infection with IB and AIV-H9 viruses, according to the results of rRT-PCR, which also showed that mixed infection is the most common cause of respiratory affection in Egypt (Table 2). Of the examined viruses, mixed infections accounted for 66.6% (20 flocks), whereas single viral infections were detected in 33.3% (10 flocks). In flocks under investigation, there found a high prevalence of AI-H9N2 and IBV (24% and 21%, respectively). The most frequent combined infection, accounting for 12 flocks or 40% of all flocks examined, was subsequently determined to be AI-H9N2 with IBV.

Table (2). Real-time RT-PCR detection of IBV, H9N2, and vNDV viruses from broiler flocks

Tested virus	Single viral infection			Mixed viral infection				Total
	IBV	H9N2	NDV	IBV-H9N2	NDV-H9N2	IBV-NDV	IBV-H9N2-NDV	
IBV	6			12	3	1	2	24 (80%)
H9N2		3		12	3		2	21 (70%)
NDV			3			1	2	8 (27%)
Total No.	6 (20%)	3(10%)	3 (10%)	12 (40%)	3 (10%)	1 (3.3%)	2 (6.6%)	30 (100%)

Molecular Identification of Avian Respiratory Viruses

Molecular analysis was used to identify eight isolates for each isolated virus, including three for IBV, two for AI-H9N2, and three for vNDV viruses. The sequences of the vNDV F gene, the hemagglutinin gene of the AI-H9N2 virus, and the IBV S1 gene were uploaded to GenBank and given accession numbers (Table 1).

Sequences encoding the S1 spike glycoprotein subunit hyper variable region (HVR 3) of IBV were examined for avian IB viruses. The collected IBV isolates were shown to be closely related to the recent Egyptian isolates of variant 2 Egyptian IBV, as determined by phylogenetic analysis (Figure 1). Our study's Egyptian IBV viruses (IBV-Eg-12120s-2012 and IBV/IS/885-00) had an identity of 85% and 91%, respectively, and were grouped within the Egy/Var-II subgroup. These results suggest that the Egyptian IBV viruses are closely linked to the variant II isolates.

IBV strains identified in this investigation displayed identities ranging from 75% to 80% for strains (H120, Ma5, D274, 4/91,1/96, and CR88121), in contrast to vaccination strains utilized in Egypt. The Var II vaccination, 1212B, displayed high similarity between 91% and 93% at the same time.

Regarding AIV-H9N2 viruses, the complete HA gene sequences of AI-H9N2 viruses revealed a relationship between all isolates that were collected and the recently circulating H9N2 strains in Egypt and the Middle East, which are members of the G1-like lineage (Group B) (Figure 3). Findings also revealed that, whereas AI-H9N2 isolates differ in terms

of amino acid identities from current vaccine strain A/Ck/Egypt/114922v/2011 by 93 to 97%, all isolates share 99.2% of these identities.

Regarding vNDV viruses. Based on their phylogenetic analysis, the three NDV isolates were identified as the highly pathogenic genotype VIIb (also known as lineage 7b class II) that caused the vNDV outbreaks in Egypt. Indicative of the velogenic nature of NDV strains, the isolates bear the distinctive cleavage site motif in the hypervariable region of the highly virulent NDV strains 112RRQKRF117. When compared to other strains listed on the NCBI website's GeneBank, all of the isolates were found to be closely related to the Chicken/China/SDWF07/2011 strain with identity, ranging from 96% to 100% (Table 3). The strains reported in this work (class II Genotype VIIb) show low degree of amino acid identity ranging from 77% to 81% in relation to the vaccinal NDV viruses (Lasota, Hitchner, clone 30 and Avinew) used in Egypt, which are genetically genotyped as lentogenic strains of NDVs (class II genotype II). On the other hand, the partial fusion amino acid sequences (Table 3) show that for the Vectormune ND vaccine (class II genotype I), the amino acid identity with genotype VIIb varied from 82% to 84%. NDV-KBNP-C4152R2L, the NDV genotype VII vaccine, has 92% amino acid identity with the strains used in this investigation (class II, genotype VIIb).

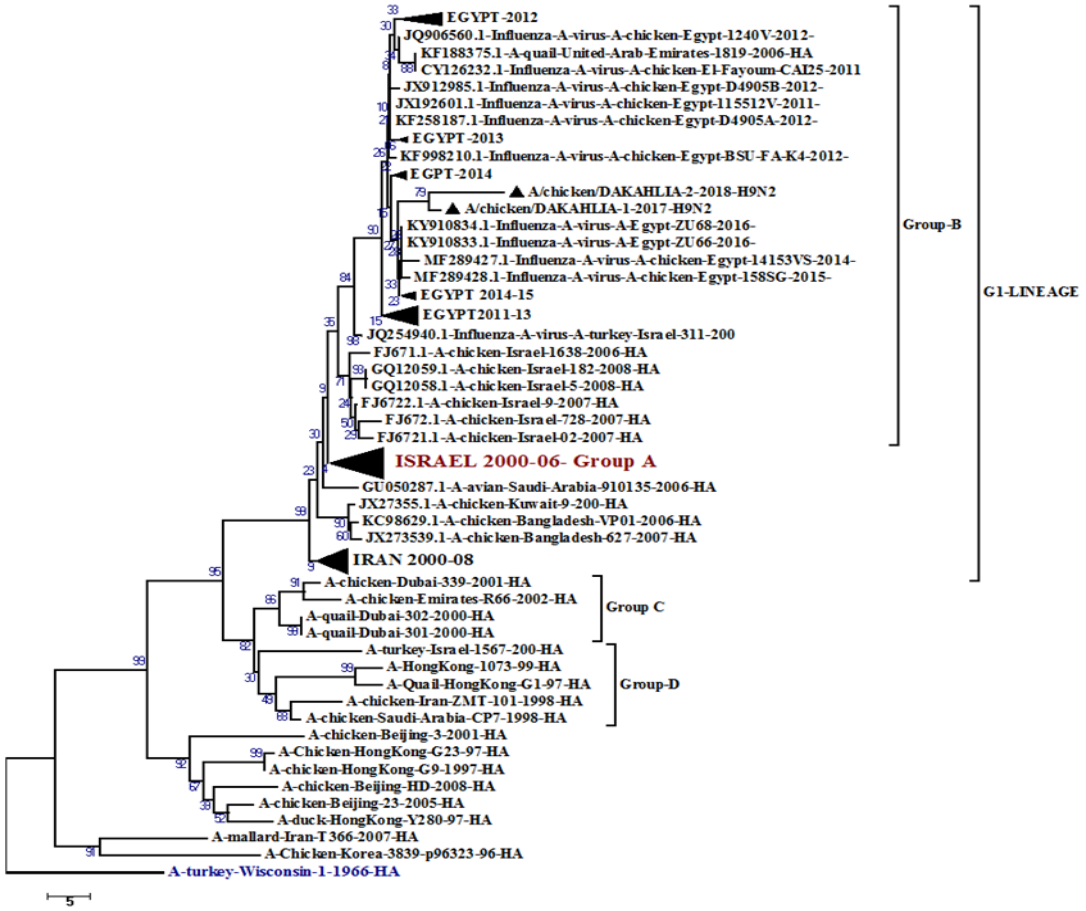


Figure (1). Using the neighbor-joining techniques of Mega 6 software, a phylogenetic tree representing the nucleotide sequences of the complete HA gene of H9N2 Egyptian isolates (shown by a solid black triangle) and the references and vaccine strains from GenBank was created.

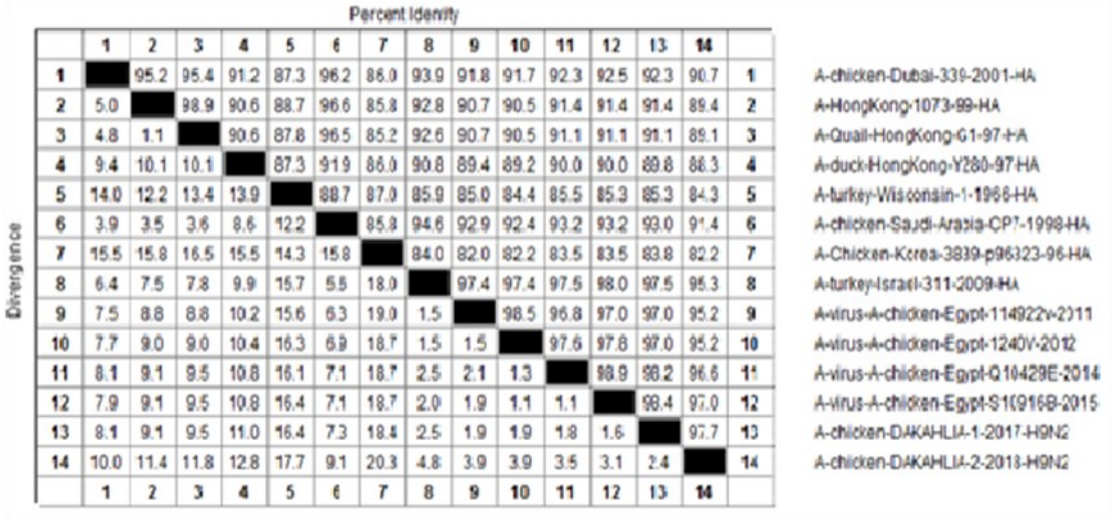


Figure (2). Deduced amino acid identities of AI-H9N2of Egyptian isolates with other selected references and vaccine strains from different serotypes

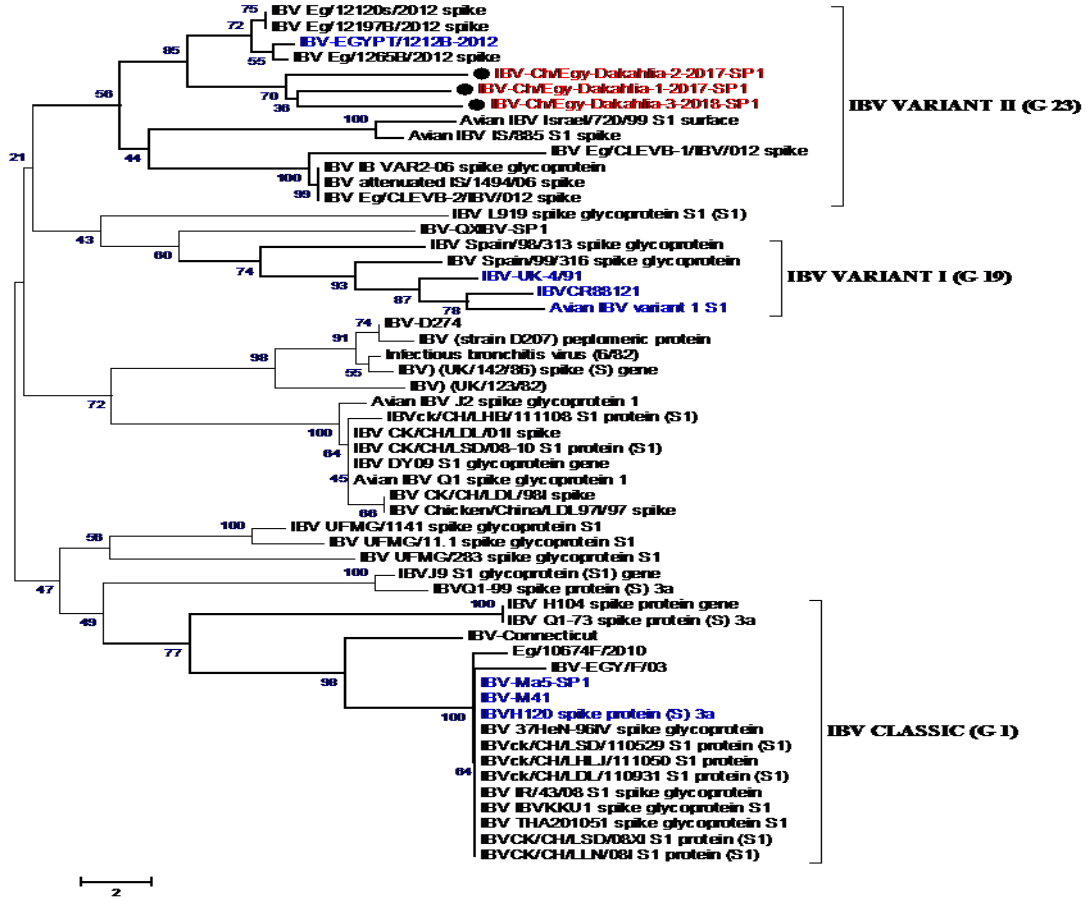


Figure (3). Phylogenetic tree of nucleotide sequences of the partial spike 1 fragment of IBV Egyptian isolates (marked with a solid black circle and red color) and the global and vaccine strains from GenBank using neighbor-joining methods of Mega6 software**.**

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1		100.0	89.6	91.5	89.6	79.2	78.3	79.2	79.2	83.0	85.8	83.0	88.7	82.1	80.2	84.9	84.9	85.8	1
2	0.0		89.6	91.5	89.6	79.2	78.3	79.2	79.2	83.0	85.8	83.0	88.7	82.1	80.2	84.9	84.9	85.8	2
3	11.2	11.2		81.1	89.6	81.1	80.2	81.1	81.1	82.1	83.0	83.0	98.1	81.1	81.1	91.5	88.7	91.5	3
4	9.0	9.0	21.8		81.1	71.7	69.8	71.7	71.7	74.5	77.4	74.5	80.2	73.6	72.6	76.4	77.4	77.4	4
5	11.2	11.2	11.2	21.8		76.4	78.3	76.4	76.4	80.2	84.0	82.1	89.6	80.2	79.2	85.8	82.1	84.9	5
6	24.4	24.4	21.8	35.5	28.4		93.4	100.0	100.0	78.3	76.4	74.5	80.2	72.6	72.6	75.5	76.4	75.5	6
7	25.7	25.7	23.1	38.6	25.7	6.9		93.4	93.4	76.4	76.4	74.5	79.2	73.6	73.6	74.5	75.5	74.5	7
8	24.4	24.4	21.8	35.5	28.4	0.0	6.9		100.0	78.3	76.4	74.5	80.2	72.6	72.6	75.5	76.4	75.5	8
9	24.4	24.4	21.8	35.5	28.4	0.0	6.9	0.0		78.3	76.4	74.5	80.2	72.6	72.6	75.5	76.4	75.5	9
10	19.3	19.3	20.5	31.2	23.1	25.7	28.4	25.7	25.7		83.0	83.0	82.1	83.0	80.2	79.2	77.4	77.4	10
11	15.7	15.7	19.3	27.0	18.1	28.4	28.4	28.4	28.4	19.3		86.8	83.0	85.8	84.9	78.3	76.4	78.3	11
12	19.3	19.3	19.3	31.2	20.5	31.2	31.2	31.2	31.2	19.3	14.6		83.0	95.3	95.3	78.3	77.4	77.4	12
13	12.3	12.3	1.9	23.1	11.2	23.1	24.4	23.1	23.1	20.5	19.3	19.3		81.1	81.1	93.4	88.7	91.5	13
14	20.5	20.5	21.8	32.6	23.1	34.0	32.6	34.0	34.0	19.3	15.7	4.9	21.8		96.2	76.4	74.5	75.5	14
15	23.1	23.1	21.8	34.0	24.4	34.0	32.6	34.0	34.0	23.1	16.9	4.9	21.8	3.9		76.4	75.5	75.5	15
16	16.9	16.9	9.0	28.4	15.7	29.7	31.2	29.7	29.7	24.4	25.7	25.7	6.9	28.4	28.4		90.6	91.5	16
17	16.9	16.9	12.3	27.0	20.5	28.4	29.7	28.4	28.4	27.0	28.4	27.0	12.3	31.2	29.7	10.1		90.6	17
18	15.7	15.7	9.0	27.0	16.9	29.7	31.2	29.7	29.7	27.0	25.7	27.0	9.0	29.7	29.7	9.0	10.1		18
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	

IBV-IS-1494-06
IBV-VAR2-06
IBV-Eg-12120s-2012
Eg-CLEVB-1-IBV-012
IBV-IS-885-S1
IBV-Ma5-SP1
IBV-Connecticut
IBV-M41
IBV-H120
IBV-D274
IBV-QXIBV-SP1
IBV-UK-4-91
IBV-EGYPT-1212B-2012
IBV-CR88121
IBV-variant-I-1-96-S1
IBV-Egy-Dakahlia-1-2017
IBV-Egy-Dakahlia-2-2017
IBV-Egy-Dakahlia-3-2018

Figure (4). Deduced amino acid identities of infectious bronchitis virus Egyptian isolates with other selected references and vaccine strains from different serotypes

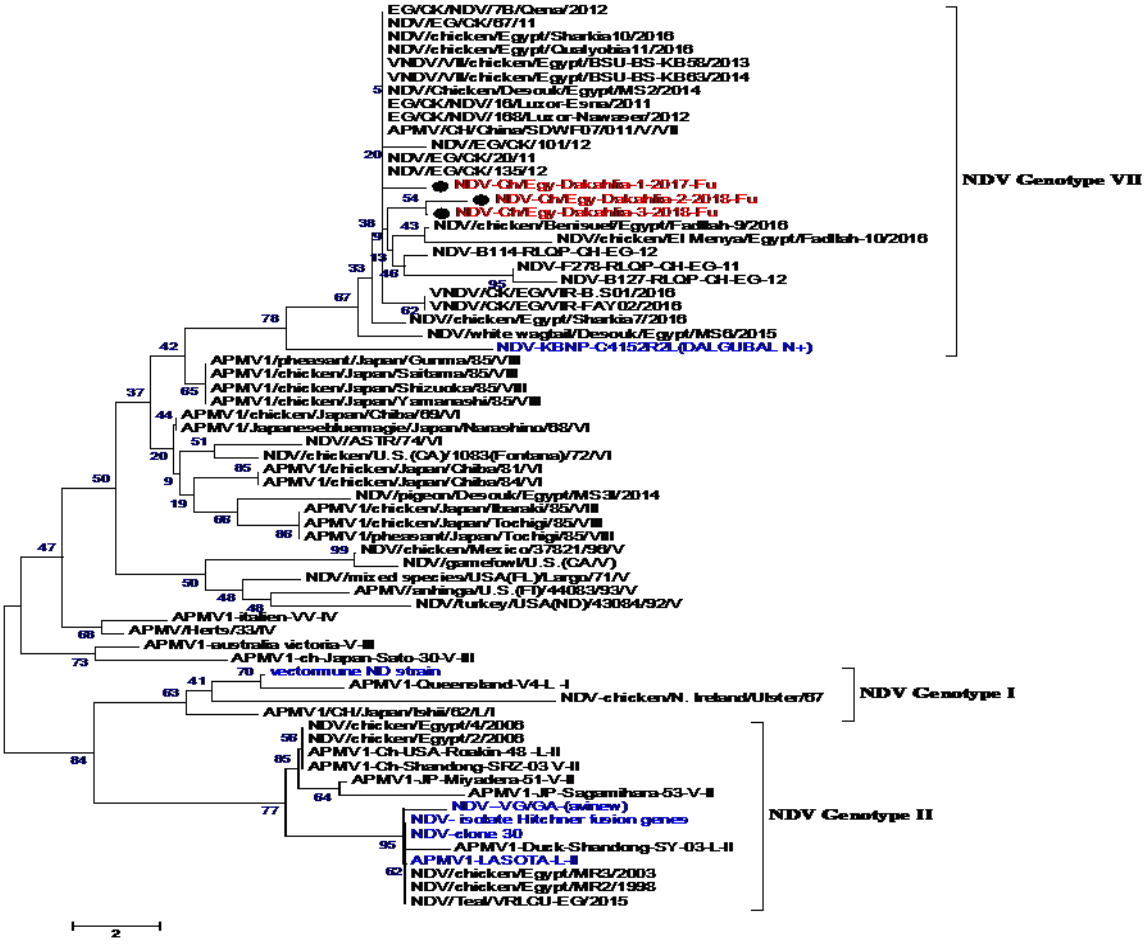
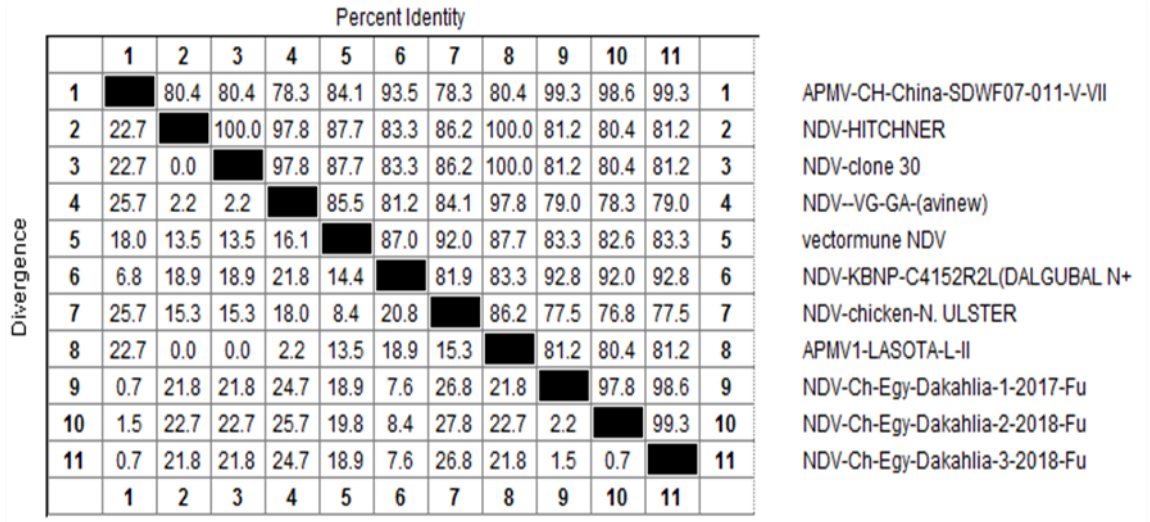


Figure (5). Phylogenetic tree of partial nucleotide sequences of the fusion gene of NDV Egyptian isolates (marked with a solid black circle and red color) and the references and Vaccinal strains from GenBank using neighbor-joining methods of Mega6 software.



	1	2	3	4	5	6	7	8	9	10	11		
1		80.4	80.4	78.3	84.1	93.5	78.3	80.4	99.3	98.6	99.3	1	APMV-CH-China-SDWF07-011-V-VII
2	22.7		100.0	97.8	87.7	83.3	86.2	100.0	81.2	80.4	81.2	2	NDV-HITCHNER
3	22.7	0.0		97.8	87.7	83.3	86.2	100.0	81.2	80.4	81.2	3	NDV-clone 30
4	25.7	2.2	2.2		85.5	81.2	84.1	97.8	79.0	78.3	79.0	4	NDV--VG-GA-(avinew)
5	18.0	13.5	13.5	16.1		87.0	92.0	87.7	83.3	82.6	83.3	5	vectormune NDV
6	6.8	18.9	18.9	21.8	14.4		81.9	83.3	92.8	92.0	92.8	6	NDV-KBNP-C4152R2L(DALGUBAL N+
7	25.7	15.3	15.3	18.0	8.4	20.8		86.2	77.5	76.8	77.5	7	NDV-chicken-N. ULSTER
8	22.7	0.0	0.0	2.2	13.5	18.9	15.3		81.2	80.4	81.2	8	APMV1-LASOTA-L-II
9	0.7	21.8	21.8	24.7	18.9	7.6	26.8	21.8		97.8	98.6	9	NDV-Ch-Egy-Dakahlia-1-2017-Fu
10	1.5	22.7	22.7	25.7	19.8	8.4	27.8	22.7	2.2		99.3	10	NDV-Ch-Egy-Dakahlia-2-2018-Fu
11	0.7	21.8	21.8	24.7	18.9	7.6	26.8	21.8	1.5	0.7		11	NDV-Ch-Egy-Dakahlia-3-2018-Fu
	1	2	3	4	5	6	7	8	9	10	11		

Figure (6). Deduced amino acid identities of Newcastle disease virus of Egyptian isolates with other selected references and vaccine strains from different serotypes

Discussion

Numerous mutually-interactive commensal and perhaps dangerous viruses reside in the respiratory systems of birds. The significant rise in avian respiratory illnesses observed globally is caused by the intensive housing practices used for chickens. Numerous viral infections have emerged and spread in tandem with the swift growth of Egypt's poultry sector and with the worldwide trade and transportation of chickens (Abdelwhab el et al., 2010). This study examined the incidence of viral infections in broiler chickens with respiratory illness issues, with an emphasis on AI, IBV, and NDV. In flocks under investigation, the largest fatalities and the development of clinical respiratory disease were typically noted as early as 20 days of age. The emergence of illness symptoms at young ages may be explained by the fact that most of the flocks under investigation were not vaccinated against AI-H9N2. Furthermore, the increased incidence of mixed respiratory virus infections suggests that AI-H9N2 may have an immunosuppressive function. Early H9N2 infection has been shown to induce immunosuppression and predispose hens to secondary infections by inducing shrinkage and lymphoid depletion of the thymus and maybe other lymphoid organs (Hadipour et al., 2011).

The current study data showed that, even in flocks that have received vaccinations, the rate of IBV infection alone is higher. Co-infection of IBV and AIV-H9 raised this rate even in flocks that had received vaccinations against both viruses. Furthermore, tracheal caseation and severe respiratory symptoms were the sole outcome of AI-H9N2 infection. According to Nili and Asasi (2003) and Haghighat-Jahromi et al. (2008), mixed infections of AI-H9N2 with other respiratory pathogens, including IBV, Mycoplasma gallisepticum, and Escherichia coli, are thought to be the cause of severe clinical disease and subsequent high mortality.

Partial protection provided by prior immunization with live lentogenic NDV vaccines may account for low detection rates in flocks infected with vNDV (Hadipour et al., 2011; Munir et al., 2012). Furthermore, the rapid and widespread transmission of AI-H9N2 LPAI may have contributed to viral interference, which could account for the low mortality rates from vNDV. A variety of processes, such as competition for attachment interference or blocking of receptor sites by the super infecting virus, intracellular competition for replication host machinery, and virus-induced interferon, can be used to explain viral interference (Ge et al., 2007). Furthermore, it was evident from the phylogenetic analysis results (Fig. 3, 4) that the sequences under study were associated with genotype VII, one of the most common and

virulent viruses that are currently in circulation worldwide (Diel et al., 2012).

According to the virus-specific rRT-PCR, co-infection with the AIH9N2 and IBV viruses, in particular, is the most common cause of respiratory illnesses. There have been prior reports on the high prevalence of AI-H9N2 (El-Zoghby et al., 2012) and IBV (AbdelMoneim et al., 2006) in Egyptian poultry. Increased rates of CO infection with both viruses suggest that IBV may play a role in making AI-H9N2 infection more severe by activating HA cleavage and supplying trypsin-like proteases that are encoded by the coronavirus (Klenk and Garten, 1994; Ng and Liu, 2000; Perk et al., 2004; Haghighat-Jahromi et al., 2008). It is important to note that the rRT-PCR assay is not able to distinguish between IBV vaccine and field strains on its own; instead, S1 gene sequencing is believed to be the sole technique that can distinguish between all IBV strains.

The three IBVs that were chosen for phylogenetic analysis were found to be closely related to one another (90–98% identity) and to be clustered within the Variant II subgroup, which includes IBV/IS/885-00 and IBV-Eg-12120s-2012, as well as other Egyptian-related strains that have been deposited in the GenBank database. Our results corroborated those of Abdel-Moneim et al. (2012), Sultan et al. (2015), Tatar-Kis et al. (2015), Zanaty et al. (2016), and EL Samadony et al. (2017), who reported that Egyptian variant II strains were the most prevalent strains recently circulated in Egypt. These findings also related to the dominance of strains belonging to Variant II in broiler flocks in Egypt during the 2017–2018.

AI-H9N2's genetic diversity may alter the virus's tissue tropism and pathogenicity (Cavanagh et al., 1992). Since 1999, the G1-like lineage of AI-H9N2 viruses, specifically groups A and B, have been widely distributed in Middle Eastern nations (Bashashati et al., 2013). Group B of the G1-like lineage includes all Egyptian isolates, including those included in our analysis (Monne et al., 2012). Despite the fact that the HA gene of the isolated H9N2 viruses exhibited minimal evolution, a small number of amino acid changes were identified, which are suggestive of antibody selection pressure on H9N2 viruses in the field. The antigenic epitope at position 127 that was previously described by Kaverin et al. (2004), receptor binding sites 128 and 180 (H9 numbering) by Wan et al. (2014), and antigenic epitope 2 at position 182 (Figure 4) were among the alterations that were noticed.

In **conclusion,** increasing severity and high death rates of respiratory infection field outbreaks in broiler chickens in Dakahlia are mostly caused by co-infection with AI-H9N2 and IBV. Variant IBV strains including many mutations at the virus neutralizing epitopes in the S1 genes were found to be circulating, and new variants were always emerging. In sensitive host populations, co-circulation of AI-H9N2 with H5N1 may have repercussions for public health. More research is needed to determine how co-infections and interference from the AI, IBV, and NDV viruses affect the severity of clinical symptoms and lesions. Finding the variables that affect the interference or synergy of the avian respiratory virus will shed light on the pathophysiology and, as a result, improve control initiatives.

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