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Molecular Detection of Respiratory Virus Infection and Co-infection in Chicken Farms

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Abstract

IN RECENT years, with the rapid expansion of poultry production and international poultry trade, the incidence of respiratory viral infections in commercial chickens in Egypt has also increased. The frequency of the most economically significant respiratory viruses circulating in chicken flocks was the primary focus of this investigation. Between 2023 and 2024, 50 flocks of chickens with respiratory, neurological, digestive tract, and kidney infections were investigated for respiratory viral pathogens using specific primers for Newcastle virus (NDV), infectious bronchitis virus (IBV), and Avian influenza virus (AIV) using real-time PCR (rtRT-PCR). Five IBV strains were selected for sequencing and identification. The rtRT-PCR test results showed that the detection rate of IBV was the highest (52%), followed by NDV (16%), and the detection rates of AIV H5 and H9 were 6% and 8%, respectively. Only two samples in this study had documented co-infection with IBV, one with AIV H9 and the other with AIV H5. The mortality rate ranged from 1.5- 40%, with a mortality rate of 16% in the case of IBV and AI-H9N2 co-infection, even with IBV vaccination, although the highest mortality rate was 40% in single NDV infection. IBV and NDV infections were detected in both vaccinated and unvaccinated flocks, whereas AIV H5 and H9 infections were detected only in unvaccinated flocks. The phylogenetic tree of the selected five IBV field strains and other reference strains showed that the five IBVs were divided into two different branches and were far away from the IB vaccine strains. Despite different vaccination programs, IBV and NDV continue to circulate and cause morbidity and mortality in chicken farms; strict hygiene measures and up-to-date vaccination regimens must be implemented to protect the chicken industry.

Keywords: Chicken, Co-infection, Infectious bronchitis, Molecular, phylogenetic tree.

Introduction

Viral respiratory infections severely impair poultry welfare and have a huge financial effect on the poultry industry, especially when multiple viruses are incriminated [1]. The most prevalent viral infections affecting poultry in Egypt are Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and avian influenza (AI) [2].

IBV is extremely contagious virus that causes severe losses to chicken flocks worldwide, typically causing respiratory symptoms, reduces egg quality and quantity, and may lead to renal failure, depending on the virus virulence [3]. IBV is a single-stranded, enveloped, positive-sense RNA pleomorphic gamma coronavirus belonging to the family Coronaviridae. The Virus virion is composed of spike (S), nucleocapsid (N), envelope (E), and membrane (M) structural proteins [4]. The S

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glycoprotein is involved in viral entry through host cell attachment and membrane fusion and the antigenic or molecular classification of virus isolates [5]. Owing to the high variability of the genome, classification has historically been complex and ambiguous, and several approaches based on the genotype, serotype, pathogenic type, and protective type have been proposed; but according to a more recent classification based on the complete Spike 1 subunit sequence, there seven genotypes (GI-GVII) [6]. The deduced amino acid sequences of the S1 subunits of different IBV serotypes differ significantly (20-50%). Cross-protection between serotypes should have more than 95% amino acid identity in the S1 subunit [7].

AIV is a re-emerging highly contagious respiratory viral disease that has devastated effect on the poultry industry and can infect humans, caused by any subtype of the avian influenza virus (H1-18, N1-11) [8]. AIV is a segmented, single-stranded, negative-sense RNA virus of the family Orthomyxoviridae that encodes at least ten viral proteins. Based on virulence, the infection can be classified as "highly pathogenic avian influenza" (HPAI), which presents a public health risk with zoonotic significance. At the same time, low pathogenic avian influenza (LPAI) is less pathogenic [9] but it has an immunosuppressive effect, leading to an increased chance of contracting other viral infections, commonly seen in H9N2 and IBV co-infections [10]. LPAI virus was first documented in Egypt in 2010-2011, isolated from commercial bobwhite quail and belongs to the G1 lineage [11]. IBV and AIV-H9N2 viruses are widely circulated and have an economic impact on poultry production because they can cause disease alone or in combination [1]. The high incidence of the co-infection of AIV-H9N2 and IBV, highlighting the possible exacerbating role of IBV in AIV-H9N2 infection in chickens, leading to higher mortality [12, 13].

In recent years, NDV has evolved, which may affect the pathogenicity of the virus, particularly the most virulent velogenic strains, which cause substantial mortality and have a significant negative economic impact on the chicken industry, despite widespread vaccination efforts in poor and tropical countries [14, 15]. NDV alone or in combination with other pathogens is the main pathogen causing increased mortality in chicken farms [16]. Despite regular vaccination, IBV and NDV still have a severe and diverse impact on Egyptian chicken production, causing high morbidity and mortality, especially when they are combined with other viruses [13, 17, 18].

Increased incidence of respiratory pathogens in poultry flocks, either individually or in combination, can have significant economic impacts. Owing to their multifaceted nature and the fact that they carry similar clinical signs, they can be misinterpreted [1].

The present study focused on molecular identification of relevant respiratory viruses circulating in chicken flocks and record their co-infection besides monitoring their breakouts in vaccinated chickens as well as exploring the relationship between circulating IBV and other Egyptian isolates, through sequencing the S1 protein.

Material and Methods

Examined chicken flocks and specimens collection

Between 2023 and 2024, 50 commercial flocks of chickens of different breeds and ages with respiratory, nervous system, digestive tract, and kidney infections were collected from Sharqa and Ismailia governorates, Egypt, for the study of respiratory viral pathogens. Clinical examinations were performed, and postmortem lesions were recorded. Tissue pools (lung, trachea, kidney, proventriculus, liver, cecal tonsils, and spleen) were collected from 3:5 chicken/ each flock of chickens and was placed in sterile plastic bags with labels, placed in an ice container and sent to the laboratory and kept at -80°C until use.

Sample processing

Frozen pooled tissue samples were thawed, frozen three times in succession, and then processed in sterile PBS to prepare tissue suspensions (10%). The homogenate was centrifuged at 3000 rpm for 15 minute, the supernatant was stored at -80 °C until used for virus detection [19].

Real time RT-PCR reaction

Using the GeneJET Viral RNA and DNA Purification Kits (Thermo Scientific, Germany), the RNA was extracted from pooled tissues homogenate according to the company's instructions. Using gene-specific primer pairs, one-step rtRT-PCR amplification of the coding parts was done to detect IBV, NDV, and AIV in all tested samples. Quantitect probe, rtRT-PCR (Qiagen, Valencia, CA, USA) was used following the manufacturer's directions (Table 2). PCR assay was performed using Quantitect master mix (Takara) code No (RR310A) with a volume of 28.5 µl, including 12.5 µl Master mix, 0.125 µl probe, 1 µl (two primers), 4.5 µl RNase-free water, 10 µl template, and 0.125 µl QuantiTect, RT Mix. Thermal cycle conditions were 50°C for 30 minutes. It was then held at 95◦C for 15 minutes, followed by 40 cycles of 94◦C for 15 seconds and 50◦C for 30 seconds, and finally an extension at 72◦C for 10 seconds [20].

Sequencing analysis

Five high Ct values (20-27) IBV samples were selected for virus characterization by sequencing.

Amplified RT-PCR products were purified following the manufacturer's directions, by QIAquick Gel Extraction Kits (QIAGEN, Hilden, Germany). Using a ready reaction Big Dye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA), purified RT-PCR product was sequenced in the forward directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Sequences were assigned to GenBank.

Phylogenetic analysis

IBV sequence analysis was performed using the CLUSTRAL W multiple sequence alignment program, the MegAlign module of the Lasergene DNAStar software Pairwise version 12.1 (Madison, WI, USA), and Phylogenetic analyses were performed using maximum likelihood, neighbor-joining, and maximum parsimony methods in MEGA6. The IBV vaccine strains and reference strains used for comparison were obtained from GenBank and are available from the National Center for Biotechnology Information (NCBI).

Results

Clinical symptoms, mortalities, and lesions

The examined flocks had respiratory, neurological and/or digestive tract and renal infections, with respiratory signs being the most prominent, such as ocular and nasal discharge, sneezing, coughing, gasping, conjunctivitis, tracheal rales, and facial edema. Mortality ranged from 1.5-40%, with IBV and AI-H9N2 co-infection having a mortality rate of 16%, even with IBV vaccination, and single NDV infection having the highest mortality rate of 40%. In exceptional cases, death can occur suddenly, without any symptoms. In addition, natural infection with AIVs does not result in a high mortality rate 4-16%.

The main lesions found in the necropsy were tracheitis, caseous plugs at the tracheal bifurcation, pneumonia, air sacculitis, and fibrinous perihepatitis. Nephritis and ureters distended with urates, congestion of the liver and internal organs, petechial hemorrhage in coronary fat, cecal tonsils and proventriculus, congestion in intestinal and brain blood vessels were observed.

Real-time PCR results for IBV, NDV and AIV in tested flocks

The results of rtRT-PCR detection are shown in Table (2) and Fig. (1), among which IBV had the highest ratio 52% (26/50), followed by NDV at 16% (8/50), and AIV H5 and H9 at 6% (3/50) and 8% (4/50), respectively. Only two samples in this study had documented co-infection with IBV, one with AIV H9 and the other with AIV H5. IBV and NDV infections were more common in vaccinated than in unvaccinated flocks, whereas AIV H5 and H9 infections were detected only in unvaccinated flocks.

The highest rate of IBV, AIV H5 and H9 infection was obtained in chicken aged between 21-30 days old, meanwhile the occurrence of NDV infection was equally detected in chicken aged between 11- 20 days and 21-30 old (Table 3).

S1 gene Sequences results of IBV

The partial sequences encoding the S1 spike glycoprotein of IBV were analyzed, and the nucleotide sequence from nucleotide position 208 to 525 of each virus was determined and used in nucleotide and amino acid analysis. The amino acid analysis showed that the amino acid identities of the five IBV strains, AS1, AS2, AS3, AS4, and AS5 (accession numbers PQ461634 to PQ461638, respectively), ranged from 82.4 to 100%. The similarity between the five IBV strains and the vaccine strain (4/91- QXIBV - H 120 - MASS) was 70.1%: 76.7 (Fig. 2).

Phylogenetic analysis showed that the five examined IBV were divided into two distinct clades. Phylogenetic analysis classified PQ461636 (AS3) and PQ461637 (AS4) as strains in the classical clade includes with Jordan, Israel, and other Egyptian isolated strains (Assuit, Sohage, Newvelly, Aswan), while PQ461634 (AS1), PQ461635 (AS2) and PQ461638 (AS5) were belonged to variant II clade with other isolated Egyptian strains (Dakahlia, Sharkia, Kafr El Sheikh, Garbia) (Fig. 3).

Discussion

Viral infectious diseases are considered to be a major problem affecting poultry production due to their high infection rate, high mortality rate, high prevention and control costs, and potential huge economic losses [21, 22]. IBV, NDV, and AIV are ranked among the most significant diseases affecting Egypt's poultry sector, whether infected alone or in combination [13]. This study evaluated the occurrence of respiratory virus infections in broiler chickens, including IBV, NDV, and AIV subtypes (H5 & H9) and record their co-infection besides monitoring their breakouts in vaccinated chickens as well as exploring the relationship between circulating IBV and other Egyptian isolates, through sequencing the S1 protein.

Clinical signs and post-mortem lesions in the examined flocks varied according to the viral infection detected. The main clinical signs in the examined flocks included nasal and ocular discharge, gasping and dyspnea or open mouth breathing in examined flocks. Neurological symptoms are occasionally observed. Tracheitis, caseous plugs at the tracheal bifurcation, pneumonia, air sacculitis, and fibrinous perihepatitis were also observed at post-mortem examination of infected cases. As well as lesions in the gastrointestinal tract, kidneys, brains, and petechial hemorrhage in coronary fat of the heart. The clinical examination results of this

study were consistent with those reported in previous studies [2, 13, 23].

The respiratory viral diseases in the tested chickens were tested by rtRT-PCR and the results showed that IBV had the highest percentage of 52% (26/50), which was almost similar to previous result [24] where IBV was recorded in 69.2% of the tested clinical samples. The severity of IBV infection in the study area indicates that IBV has been widespread and prevalent in Egypt since the virus was first described and isolated [25, 26]. Primers used to detect IBV by rtRT-PCR target the highly conserved nucleocapsid (N) gene of IBV, which is highly specific for IBV but cannot distinguish between IBV strains (vaccine and wild-type), but clinical signs were observed between examined flocks side by side with the results of sequencing and the phylogenetic analysis for five IBV field strains indicated that these strains were far from IBV/H120, IBV/Ma5, IBV/M41 and IBV/4/91 vaccine and related to IBV Egyptian variants-II and classical strains. Therefore, the detection of IBV in vaccinated flocks indicates that these flocks had exposed to field IBV and excludes the possibility to be due to vaccinal strain [27].

The IBV infection was recorded in both vaccinated and unvaccinated flocks, IBV outbreaks have been observed in chicken farms despite the use of different vaccines (H120 and 4/91) [28, 29]. The higher IBV exposure rate in vaccinated flocks may be due to the inability of IBV vaccines to provide effective cross protection against different serotypes [30] as well as the emergence of new IBV variants recently in Egypt [31]. IBV control in Egypt remains a formidable challenge as different serotypes of IBV circulate in Egyptian poultry farms and the emergence of new strains poses a risk of IBV vaccination failure. In addition, some production-intensive areas lack consistent biosecurity levels, and there is a possibility of co-infection with other respiratory viruses.

The occurrence of NDV in this study was 16% (8/50), while AIV H5 and H9 recorded 6% (3/50) and 8% (4/50), respectively. Our results agree with data obtained by Shalaby et al., [32] who reported that 21% of the examined flocks were positive during NDV surveillance in Egypt in 2011and 2012. Despite the different vaccination programs against the NDV disease, it is still one of the most prevalent avian diseases. Additionally, Abdelwhab et al., [33] reported that 12.4% of tested flocks were positive for AIV (H5). In recent years, HPAI have continued to spread around the world, causing serious public health problems and huge economic losses. Control efforts should focus on areas where the risk of transmission remains high [34].

Only two samples in this study had documented co-infection with IBV, one with AIV H9 and the other with AIV H5. The most common causes of respiratory coinfection in Egypt are IB virus and AIV-H9 virus [13], the mechanism of this finding may be that IBV-induced tracheitis is associated with loss of goblet cells and the cilia, increasing epithelial susceptibility to secondary viral infection [35].

In the flocks investigated, the peak incidence of most respiratory viral diseases generally occurred between 21 and 30 days of age [13]. Since this age is favorable for virus survival, high ammonia levels, poor ventilation in different farms and associated with the increased capacity of poultry breeding in Egypt. Mortality ranged from 1.5-40%, with IBV and AI-H9N2 co-infection having a mortality rate of 16%, and single NDV infection having the highest mortality rate of 40% even with vaccination. Considering that only about 60% of the chickens can be protected by mass vaccination, the rest of the chickens having insufficient immunity [36]. It is important to note that under field conditions, multiple factors may contribute to reduced vaccination effectiveness, such as in accurate regimen and inappropriate dosing, nutritional deficiencies, and the presence of immunosuppressive diseases. Significant impact of IBV, AIV and NDV not only is the high mortality rate, but it also increases the likelihood of subsequent respiratory bacterial infections [37, 38].

The rtRT-PCR testing alone cannot distinguish different IBV strains, and sequencing, especially S1 gene sequencing, is considered to be the only way to distinguish all IBV strains. Consequently, partial sequencing and phylogenetic of IBV S1 gene of 5 field strains were analyzed and it was revealed that two strains PQ461636 (AS3) and PQ461637 (AS4) were closely related to the classical clade includes with Jordan, Israel, and other Egyptian isolated strains (Assuit, Sohage, Newvelly, Aswan). And three strains PQ461634 (AS1), PQ461635 (AS2) and PQ461638 (AS5) were belonged to Egyptian variant II that were firstly isolated in Egypt during the late 2011 [31].

The phylogenetic analysis indicated that the 5 chosen field strains were far from IBV/H120, IBV/QX, and IBV/4/91 vaccine. The 5 field strains diverged in amino acid sequences by (23.3- 29.9%) from IBV/H120, IBV/QX, and IBV/4/91 vaccine. This indicates significant differences between circulating IBV strains and IBV vaccine strains and suggests that these vaccines provide less protection against field infection. It may highlight the need for a re-evaluation of the IBV commercial vaccines currently being used in Egypt [39].

Conclusion

Respiratory viral diseases such as IBV and NDV continue to circulate in poultry farms despite different vaccination programs resulting in high morbidity and mortality rates. Whereas AIV H5 and

H9 infections were detected only in unvaccinated flocks. Poultry farms must also implement additional biosecurity measures and constantly review and update their vaccines to target mutant strains of avian respiratory viruses.

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Declaration of Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical of approval

The collection, transportation, and handling of samples in this study were in accordance with the regulations of the Scientific Research and Bioethics Committee of the Faculty of Veterinary Medicine of Suez University, Ismailia, Egypt, approval number (2018117).

TABLE1. RT-PCR primers for examined viruses

Gene	Primer	Sequence (5′-3′)	References
N gene	IBV-fr	ATG CTC AAC CTT GTC CCT AGC A	[40]
	IBV-as	TCA AAC TGC GGA TCA TCA CGT	
	IBV-TM	(FAM-TTG GAA GTA GAG TGA CGC CCA AAC TTC A-TAMRA)	
F gene	NDV-fr	TCC GGA GGA TAC AAG GGT CT	[41]
	NDV-as	AGC TGT TGC AAC CCC AAG	
	NDV-TM	[FAM]AAG CGT TTC TGT CTC CTT CCT CCA [TAMRA]	
H5 gene	AIV H5-fr	ACA TAT GAC TAC CCA CAR TAT TCA G	[42]
	AIV H5-as	AGA CCA GCT AYC ATG ATT GC	
	AIV H5-TM	6-FAM-TCW ACA GTG GCG AGT TCC CTA GCA-TAMRA	
H9 gene	AIV H9-fr	GGA AGA ATT AAT TAT TAT TGG TCG GTA C	[43]
	AIV H9-as	GCC ACC TTT TTC AGT CTG ACA TT	
	AIV H9-TM	6-CY5-AAC CAG GCC AGA CAT TGC GAG TAA GAT CC- TAMRA	

TABLE 2. Detection of IBV, NDV, AIV and co-infections in chicken flocks using rtRT-PCR

Tested virus	Number of examined flocks	Positive flocks using rtRT-PCR	
		No	Percent
IBV	50	26	52%
NDV	50	8	16%
AIV H5	50	3	6%
AIV H9	50	4	8%
IBV and AIVH5 Coinfection	50	1	2%
IBV and AIVH9 Coinfection	50	1	2%

TABLE 3. Occurrence of IBV, NDV and AIV infection of examined flocks in relation to age

Age/days	% of Positive flocks using rtRT-PCR			
	IBV	NDV	AIVH5	AIVH9
0- 10	0/26 (0%)	0/8 (0%)	0/3 (0%)	1/4 (25%)
11- 20	7/26 (26.9 %)	4/8 (50%)	0/3 (0%)	1/4 (25%)
21- 30	18/26 (69.2 %)	4/8 (50%)	3/3 (100%)	2/4 (50%)
31- 40	1/26 (3.8 %)	0/8 (0%)	0/3 (0%)	0/2(0%)

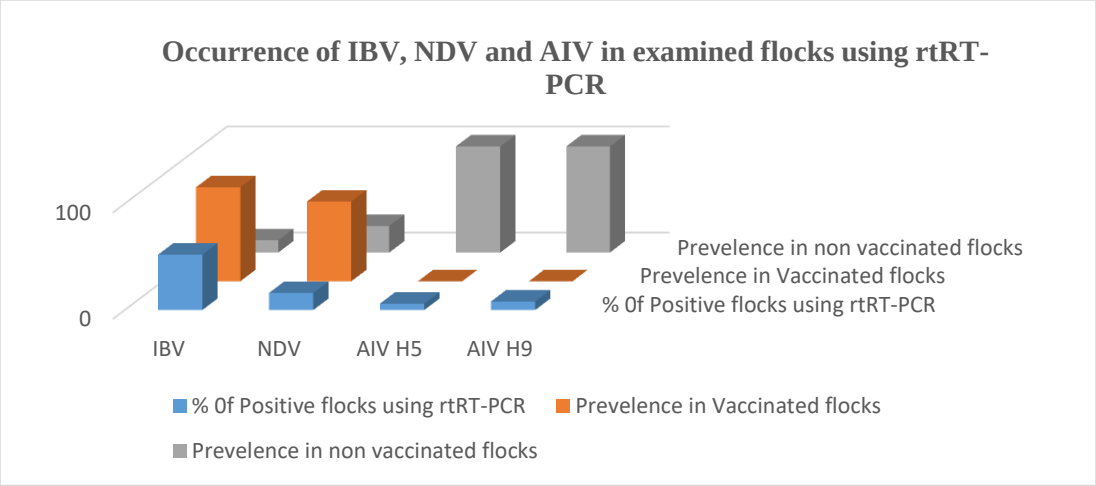

Fig. 1. Occurrence of IBV, NDV and AIV in examined flocks using rtRT-PCR

Percent Identity

Divergence

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
	71.1	75.5	99.4	70.1	70.1	70.4	70.1	70.8	70.1	70.1	71.1	71.1	75.5	75.5	75.5	74.5	75.2	75.5	74.2	75.5	73.9	74.5	70.1	71.1	75.5	75.2	70.1	1	EU822341 H120
34.7		76.1	71.4	74.2	74.2	74.2	74.5	75.8	74.2	74.2	74.2	74.2	76.7	76.7	76.4	77.4	77.4	76.7	74.8	76.7	76.4	75.5	74.2	74.2	76.7	76.4	74.2	2	KF377577 4/91 vaccine
27.7	28.8		76.1	71.1	73.9	73.9	74.2	74.5	73.9	73.9	73.9	73.9	73.3	73.0	73.3	73.0	73.0	73.3	71.7	73.0	73.3	72.0	73.9	73.9	73.3	72.6	73.9	3	MG272490 QXIBV 724/2017
0.6	34.2	26.8		70.8	70.8	71.1	70.8	71.4	70.8	70.8	71.7	71.7	76.1	76.1	76.1	75.2	75.8	76.1	74.8	76.1	74.5	75.2	70.8	71.7	76.1	75.8	70.8	4	MK248868 IBV_Mass
36.0	31.7	36.6	35.0		72.3	73.0	73.3	73.9	72.3	72.3	71.7	71.7	76.7	76.4	76.4	76.4	76.1	76.7	75.8	76.4	75.8	76.1	72.3	71.7	76.7	76.1	72.3	5	AF395531 Egy/Beni-Seuf/01
36.6	32.1	32.2	35.5	35.0		98.1	98.7	97.2	99.4	100.0	93.1	93.1	82.4	83.6	82.7	83.3	82.7	82.4	82.7	83.6	84.3	83.6	100.0	93.1	82.4	83.3	100.0	6	KU979007 EG/1212B-SP1-2012
35.9	32.1	32.2	34.8	34.0	1.9		99.4	96.9	98.1	98.1	93.1	93.1	83.6	84.9	83.3	84.6	84.0	83.6	84.0	84.9	84.9	84.9	98.1	93.1	83.6	84.6	98.1	7	OR515486 CK/Jordan/MQA-N-7/2021
36.5	31.5	31.7	35.4	33.5	1.3	0.6		97.2	98.7	98.7	93.1	93.1	83.6	84.9	83.3	84.6	84.0	83.6	84.0	84.9	84.9	84.9	98.7	93.1	83.6	84.6	98.7	8	OR515485 CK/Jordan/MQA-N-6/2021
35.3	29.6	31.2	34.2	32.4	2.9	3.2	2.9		96.5	97.2	91.5	91.5	83.6	84.9	83.3	84.9	84.6	83.6	84.0	84.9	84.9	84.9	97.2	91.5	83.6	84.6	97.2	9	MW233647 CK/sharkia/FVCU/2019
36.7	32.1	32.3	35.6	35.0	0.6	1.9	1.3	3.6		99.4	93.1	93.1	83.0	84.3	83.3	84.0	83.3	83.0	83.3	84.3	84.9	84.3	99.4	93.1	83.0	84.0	99.4	10	OP585569 F1282-9-IB-2022
36.6	32.1	32.2	35.5	35.0	0.0	1.9	1.3	2.9	0.6		93.1	93.1	82.4	83.6	82.7	83.3	82.7	82.4	82.7	83.6	84.3	83.6	100.0	93.1	82.4	83.3	100.0	11	PQ093653 Sohag-13-EGYIBV-GI23-2024
35.0	32.1	32.2	33.9	36.1	7.3	7.3	7.3	9.1	7.3	7.3		100.0	84.9	85.5	84.6	85.5	85.2	84.9	85.2	85.5	85.8	85.5	93.1	100.0	84.9	85.2	93.1	12	PQ093656 Assuit-1-EGYIBV-GI12-D274-2023
35.0	32.1	32.2	33.9	36.1	7.3	7.3	7.3	9.1	7.3	7.3	0.0		84.9	85.5	84.6	85.5	85.2	84.9	85.2	85.5	85.8	85.5	93.1	100.0	84.9	85.2	93.1	13	PQ093657 Sohag-/GI-12/D274-35/2023
27.7	28.2	33.2	26.8	28.1	20.3	18.7	18.7	18.6	19.4	20.3	17.0	17.0		98.7	99.7	97.5	98.4	100.0	96.9	98.7	93.1	97.5	82.4	84.9	100.0	98.4	82.4	14	OP585566 F1282-6-IB-2022
27.7	28.1	33.7	26.8	28.6	18.6	17.0	17.0	17.0	17.8	18.6	16.2	16.2	1.3		98.4	98.1	97.8	98.7	97.8	100.0	93.7	98.7	83.6	85.5	98.7	99.7	83.6	15	OR233127 CK/Israel/329766/2019
27.7	28.6	33.2	26.8	28.6	19.9	19.1	19.1	19.0	19.0	19.9	17.4	17.4	0.3	1.6		97.2	98.1	99.7	96.5	98.4	92.8	97.2	82.7	84.6	99.7	98.1	82.7	16	OP585570 F1282-10-IB-2022
29.1	27.1	33.7	28.2	28.6	19.0	17.4	17.4	17.0	18.2	19.0	16.2	16.2	2.6	1.9	2.9		96.5	97.5	96.2	98.1	93.7	96.9	83.3	85.5	97.5	97.8	83.3	17	PQ093639 BeniSeuf-3-EGYIBV-GI23-2024
28.2	27.2	33.7	27.2	29.1	19.8	18.2	18.2	17.4	19.0	19.8	16.6	16.6	1.6	2.2	1.9	3.6		98.4	95.9	97.8	92.1	96.5	82.7	85.2	98.4	97.5	82.7	18	OQ730214 EGY-ZU/CK/Dak-127/2021
27.7	28.2	33.2	26.8	28.1	20.3	18.7	18.7	18.6	19.4	20.3	17.0	17.0	0.0	1.3	0.3	2.6	1.6		96.9	98.7	93.1	97.5	82.4	84.9	100.0	98.4	82.4	19	OP585563 F1282-3-IB-2021
29.6	30.9	35.7	28.7	29.6	19.8	18.2	18.2	18.2	19.0	19.8	16.6	16.6	3.2	2.2	3.5	3.9	4.2	3.2		97.8	91.8	99.1	82.7	85.2	96.9	97.5	82.7	20	PQ093645 Sohag-5-EGYIBV-GI23-2023
27.7	28.1	33.7	26.8	28.6	18.6	17.0	17.0	17.0	17.8	18.6	16.2	16.2	1.3	0.0	1.6	1.9	2.2	1.3	2.2		93.7	98.7	83.6	85.5	98.7	99.7	83.6	21	MN651567 EGY/CH/F564-2019
30.2	28.6	33.2	29.2	29.6	17.9	17.1	17.1	17.1	17.1	17.9	15.9	15.9	7.3	6.6	7.6	6.6	8.3	7.3	8.7	6.6		92.5	84.3	85.8	93.1	93.4	84.3	22	OR233143 CK/Israel/410865/2022
29.1	30.0	35.2	28.2	29.1	18.6	17.0	17.0	17.0	17.8	18.6	16.2	16.2	2.6	1.3	2.9	3.2	3.6	2.6	0.9	1.3	8.0		83.6	85.5	97.5	98.4	83.6	23	PQ093652 Newvalley-12-EGYIBV-GI23-2024
36.6	32.1	32.2	35.5	35.0	0.0	1.9	1.3	2.9	0.6	0.0	7.3	7.3	20.3	18.6	19.9	19.0	19.8	20.3	19.8	18.6	17.9	18.6		93.1	82.4	83.3	100.0	24	PQ461634 AS1
35.0	32.1	32.2	33.9	36.1	7.3	7.3	7.3	9.1	7.3	7.3	0.0	0.0	17.0	16.2	17.4	16.2	16.6	17.0	16.6	16.2	15.9	16.2	7.3		84.9	85.2	93.1	25	PQ461635 AS2
27.7	28.2	33.2	26.8	28.1	20.3	18.7	18.7	18.6	19.4	20.3	17.0	17.0	0.0	1.3	0.3	2.6	1.6	0.0	3.2	1.3	7.3	2.6	20.3	17.0		98.4	82.4	26	PQ461636 AS3
28.2	28.6	34.2	27.3	29.1	19.0	17.5	17.5	17.4	18.2	19.0	16.6	16.6	1.6	0.3	1.9	2.2	2.6	1.6	2.6	0.3	6.9	1.6	19.0	16.6	1.6		83.3	27	PQ461637 AS4
36.6	32.1	32.2	35.5	35.0	0.0	1.9	1.3	2.9	0.6	0.0	7.3	7.3	20.3	18.6	19.9	19.0	19.8	20.3	19.8	18.6	17.9	18.6	0.0	7.3	20.3	19.0		28	PQ461638 AS5
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		

Fig. 2. Identity matrix for the IBV sequence strained compared with vaccinal, Egyption and other strains obtained from ncbi

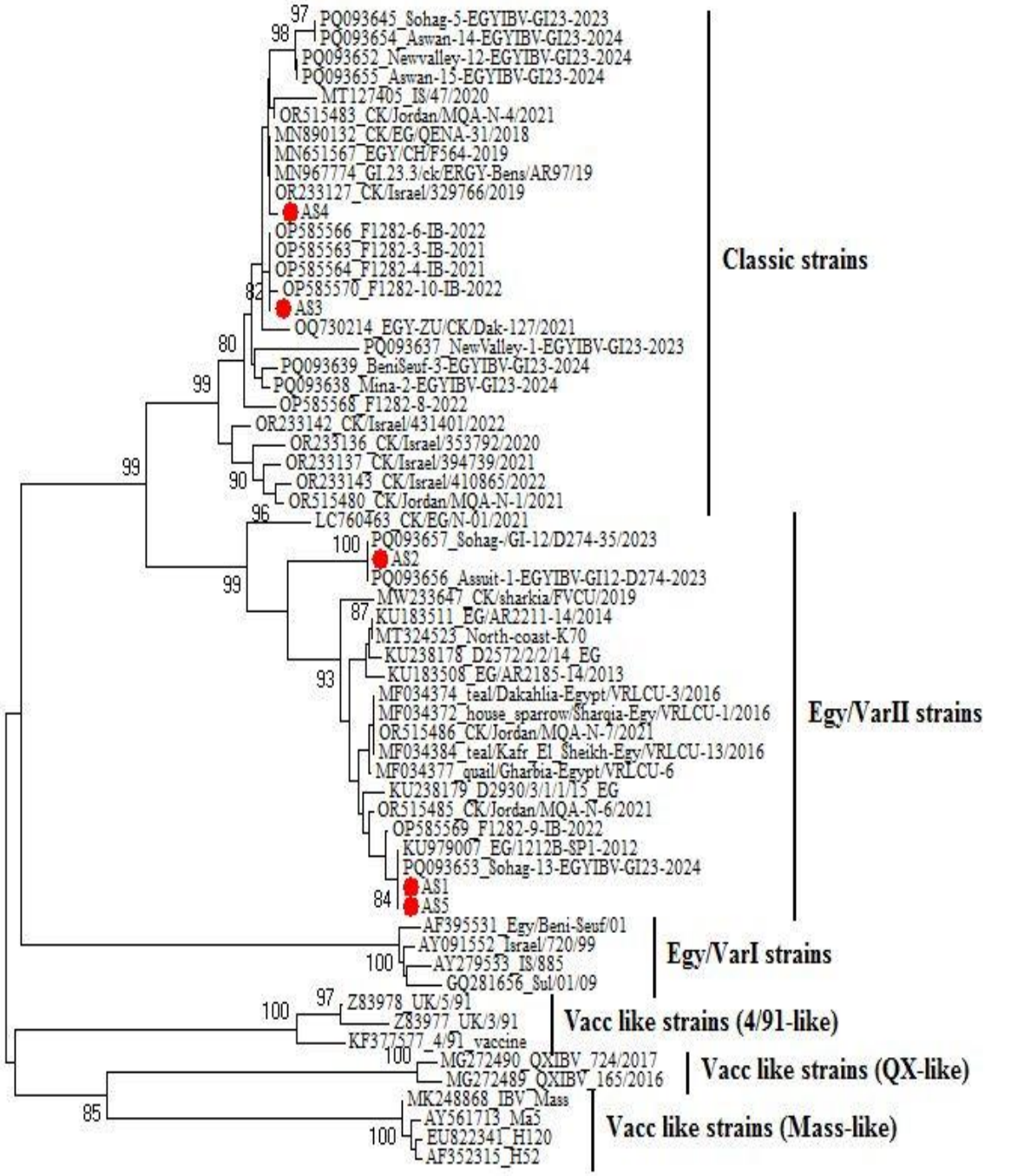

Fig. 3. Phylogenetic tree based on a partial sequence of IBV. Showing the relationship between the selected IBV isolates in the present study in comparison to reference and vaccine strains **from gene bank. Red circles refer to chosen isolates in current study.**

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الكشف الجزيئي عن عدوى فيروس الجهاز التنفسي والعدوى المشتركة في مزارع الدجاج

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الملخص

في السنوات الأخيرة، ومع التوسع السريع في إنتاج الدواجن والتجارة الدولية للدواجن، ازدادت أيضًا حالات الإصابة بالعدوى الفيروسية التنفسية لدى الدجاج التجاري في مصر. وقد ركزت هذه الدراسة بشكل رئيسي على تواتر انتشار أهم فيروسات الجهاز التنفسي من الناحية الاقتصادية بين قطعان الدجاج. بين عامي 2023 و2024، تم فحص 50 قطيعًا من الدجاج مصابًا بعدوى الجهاز التنفسي والعصبي والجهاز الهضمي والكلى بحثًا عن مسببات الأمراض الفيروسية التنفسية باستخدام بادئات محددة لفيروس نيوكاسل، وفيروس التهاب الشعب الهوائية المعدي ، وفيروس إنفلونزا الطيور باستخدام تقنية تفاعل البوليميراز المتسلسل اللحظي. تم اختيار خمس سلالات من فيروس التهاب الشعب الهوائية المعدي للتسلسل. أظهرت نتائج اختبار تفاعل البوليميراز المتسلسل اللحظي أن معدل اكتشاف فيروس التهاب الشعب الهوائية المعدي كان الأعلى بنسبة 52%، يليه فيروس النيوكاسل بنسبة 16% ، بينما بلغ معدل اكتشاف فيروسي إنفلونزا الطيور H5 و H9 6% و8% على التوالي. تراوح معدل الوفيات بين (1.5- 40%)، وكانت معدل الوفيات 16% في حالة الإصابة المشتركة بين التهاب الشعب الهوائية المعدي وفيروس انفلونزا الطيور-AI) H9N2 ، بينما كانت أعلى معدل وفيات 40% في حالة الإصابة بفيروس النيوكاسل المفرد. تم الكشف عن إصابات بفيروس التهاب الشعب الهوائية المعدي وفيروس النيوكاسل في كل من القطعان الملقحة وغير الملقحة، بينما تم الكشف عن إصابات فيروس انفلونزاالطيور H5 و H9 فقط في القطعان غير الملقحة. أظهرت الشجرة التطورية لسلالات فيروس التهاب الشعب الهوائية المعدي الخمسة المختارة وسلالات مرجعية أخرى أن سلالات فيروس التهاب الشعب الهوائية المعدي قد انقسمت إلى فرعين مختلفين وكانت بعيدة كل البعد عن سلالات اللقاحات. على الرغم من برامج التطعيم المختلفة، لا يزال فيروس التهاب الشعب الهوائية المعدي وفيروس النيوكاسا ينتشران ويسببان معدلات اعتلال ووفيات في مزارع الدجاج؛ يجب تطبيق تدابير صحية صارمة وأنظمة تطعيم حديثة لحماية صناعة الدجاج.

الكلمات المفتاحية: دجاج, عدوى مشتركة؛ التهاب الشعب الهوائية المعدي, جزيئي, الشجرة التطورية.