 Egypt. J. Vet. Sci. **Vol. 55,** No. 1, pp. 223-232 (2024)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Molecular Genotyping and Pathogenicity Study for Avian Infectious Bronchitis Virus Currently Epidemic in Chicken Flocks in North Egypt during 2021

Sameh Abdel-Moez Amer*, Hagar Magdy Ahmed and Asmaa Mahmoud Maatouq
Department of Poultry Diseases, Veterinary Research Institute, National Research Centre, P.O. Code 12622, Dokki, Cairo, Egypt.

INFECTIOUS bronchitis virus (IBV) became endemic in Egypt with multiple outbreaks in both broiler and layer chickens despite the heavy vaccination regimens. The aim of the current study is to determine the genotypic and pathotypic characteristics of the currently circulated IBVs in different Egyptian governorates. Thirty chicken flocks with a vaccination history against IB were screened using egg propagation and a reverse transcriptase-PCR assay targeting the spike protein gene (S) and further full-length S1 gene sequence analysis was also carried out. As well, the pathotypic features of the obtained IBV isolates were assessed by challenge trails in 25 days-old commercial broilers. The results revealed that, the obtained four positive IBV isolates *IBV-CH-EG-QAL-VVT.NRC-2021, IBV-CH-EG-ALEX-VVT.NRC-2021, IBV-CH-EG-GH-VVT.NRC.2021* and *IBV-CH-EG-BH-VVT.NRC-2021* were all clustered in the distinct genotype I.23 lineage (GI.23) and phylogenetically within the Egyptian variant group the so-called Egyptian variant 2 (Egy/Var2) related to others previously registered in Egypt and neighboring countries. Furthermore, the 4 isolates were found to be pathogenic to broiler chickens with various mortalities of 30 and 40% along with respiratory and nephropathogenic lesions. These findings indicated the prevalence of IBV variant strains in chicken flocks in Egypt with highly pathogenic features that heavily strike the poultry industry with severe economic losses even in vaccinated flocks.

Keywords: Infectious bronchitis virus, Genotypic, Pathotypic, Full-length S1 gene, Egyptian variant 2.

Introduction

IB is a highly infectious respiratory viral pathogen of poultry and belongs to the order Nidovirales, family Coronaviridae and subfamily coronavirinae in a Gammacoronavirus genus [1]. The IBV is a single stranded, positive sense RNA virus with of 27 kb genome that encodes 4 constructional proteins including; the nucleocapsid (N) proteins, the membrane (M), the envelope (E) and the spike (S) glycoprotein. The protein S induces neutralizing, haemagglutination-inhibition antibodies and serotype- specific viral epitopes as well as encourages also for the binding of the virus to the infected cell via cleavage with proteolysis to S1 and S2 subunits [2].

IB infects all ages of chickens producing respiratory, renal and reproductive forms in broilers and layer chickens with premium morbidity and mortality rates based on strain virulence, age of bird and presence of co-infectors either bacterial or viral diseases [3].

The genotypic classification of IBVs based on full-length S1 sequencing denotes IBV contains 6

*Corresponding author: Sameh Abdel-Moez Amer, E-mail: drsamehnrc@hotmail.com, Tel: 01019022002
(Received 27/08/2023, accepted 22/09/2023)
DOI: 10.21608/EJVS.2023.232254.1578
©2024 National Information and Documentation Center (NIDOC)

genotypes of 32 lineages in which genotype I is the only that has 27 distinguished viral lineages (GI.1 to GI.27) while other genotypes has only one viral lineage [4]. Such viral lineages are geographically prevalent in definite localities as in the Middle East (GI-23), Africa (GI-26), Europe (GI-21 and GII-1), Asia (GI-7, -15, -18, -22, -24 and GVI-1), North America (GI-8, -9, -17, -20, -25, -27 and GIV-1) and South America (GI-11) [5].

In Egypt, the first statement for IBV isolation and identification was reported in 1954 [6]. Since that time various IBV isolates have been recognized related to the classic M41 strain and Dutch D274 and D1466 variant strains [7]. Later on in 2001, GI.23 was the most predominantly circulated in Egypt and Middle East and commonly called Egyptian variant 1 (Egy var 1), and thereafter during 2012 a new clade was emerged within GI.23 and so-called Egyptian variant 2 (Egy var 2) [8].

IBV is quietly prevalent in the poultry field and had a major impact on the poultry industry in Egypt, in where several variants were emerged periodically and co-circulated within chicken flocks. Furthermore, the routine vaccination method for disease prevention was historically based on classic IBV strains H120 and Ma5, but by 2012 a new variant vaccinal strains like 4/91, CR88 and D274 were implemented in the poultry sector along with the classic vaccines to overcome the frequent IB outbreaks in Egypt (9).

Therefore, the main aim of this work is the genotyping and pathotyping of the currently prevalent variant strains of IBV within chicken flocks in different Egyptian governorates and compares them with the formerly isolated strains from Egypt and other nearby countries.

Material and Methods

Ethical approve

The birds sampling and pathogenicity experiment were carried out in accordance with the strict policies regarding animal care and handling, that were approved with a registration number of; 34712012022 by the Medical Research Ethical Committee (MREC) at the National Research Centre, Dokki, Cairo, Egypt.

Sample collection and surveillance

IB was surveyed from thirty chicken farms including 15 commercial broilers, 10 layers and 5 native breed flocks with intensive density in four different Egyptian governorates (Alexandria, El Behera, El Gharbia and Qalyubia) during the year 2021 from January till august months. The samples were collected from birds suffering from respiratory illness, depression, decreased feed and water intake, renal lesions and raised mortalities or even drop in egg production even though they had a vaccination chronicle against IB. Tracheal and kidney samples were collected from freshly dead birds in the 30 farms and pooled together in 30 samples (one trachea and one kidney sample in a pool) under sterile conditions and transported in a medium contains phosphate buffer saline (PBS) supplied with 1% penicillin and streptomycin mixture and stored in -60C for future isolation and propagation of the virus.

Virus propagation and isolation

The obtained tissue homogenate was mixed to PBS PH 7.2 containing 1% Antibiotics of Penicillin and streptomycin. The suspension was centrifuged at 5000 rbm for 10 minutes and then a volume of 100 µl from each tissue homogenate was inoculated into the allantoic sac of five specific pathogen free (SPF) embryonated chicken eggs (ECE) 10 days-old as the first blind passage. The injected eggs were incubated at 37 C and tested daily with candling for embryo deaths and after five days the allantoic fluids were aspirated and then prepared again for another set of four successive passages as described earlier (10).

Purification of IBV from mixed infection

The harvested allantoic fluids were washed and treated with red blood cell (RBCS) suspension to avoid probable co-infection with haemagglutinating viruses (HA). RBCS were collected in sterile anticoagulant (Alsever solution) and immediately washed by sedimentation and resuspension. The allantoic fluids were mixed together with RBCS suspension and incubated at 37 C for 24 hours, then the mixture was centrifuged at 5000 rpm for 10 min and the supernatant was aspirated and tested for HA activity via HA test. The previous set was repeated for 3 another successive trials to ensure get rid of HA agents or viruses (10). Finally the treated allantoic fluids of the study isolates were tested with RT-PCR for HA viruses such as Newcastle disease virus (NDV) and avian influenza (H5 and H9).

Conventional RT-PCR screening

Among the 30 collected allantoic fluid samples, viral RNA was extracted using a QIAamp viral RNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Polymerase chain reaction was carried out using a set of primers.

The S1 gene of IBV was targeted and amplified using a sense primer 5'-AAG ACT GAA CAA AAG ACC GAC T-3' and an antisense primer 5'-CAA AAC CTG CCA TAA CTA ACAT A-3' producing an amplified segment of about 1760 bp of the whole S1 gene. The thermocycler was set at 35 cycles. During the process, the cDNA was denaturated at 98◦C for 10 s, annealed at 55◦C for 5 s, and extended at 72◦C for 20 s. After the 35 cycles, a final 10 min DNA extension step was conducted at 72◦C according to **(11)**. The RT-PCR product was then visualized by 1.5% agarose via gel electrophoresis assay.

S1 gene sequencing and phylogenetic analysis

The PCR products were purified using PCR purification Kit (Qiagen, Valencia, CA) in agreement with Manufacturer procedures. The IBV S1 gene amplicons of the present study isolates were sequenced using Sanger dideoxy method with the specific primers and nucleotide sequences were submitted in Genbank (http://www.ncbi.nlm.nih.gov/Genbank) with their accession numbers tabulated in (Table.1). The S1gene obtained sequences were aligned and blasted by employing Clustal W method DNASTAR® Lasergene software and exported as FASTA files using Bioedit software that all files were used as a construction for phylogenetic analysis using Maximum likelihood method with various IBV genotypes including variant and vaccinal strains that were downloaded from Genbank.

Pathogenicity challenge experiment

Fifty one day-old commercial broiler chicks were isolated in specific isolators and divided into five groups of ten of birds each which fed and reared ad-libitum. At 25 days of age birds in group 1.2.3 and 4 were challenged with isolates; *IBV-CH-EG-QAL-VVT.NRC-2021, IBV-CH-EG-ALEX-VVT.NRC-2021, IBV-CH-EG-GH-VVT.NRC.2021* and *IBV-CH-EG-BH-VVT.NRC-2021* respectively for each group via occulonasal route by median embryo infective dose (EID50) 10^5 / ml. while chickens in group 5 was mock-inoculated with sterile PBS and act as negative non-challenged control group. All birds were monitored for 7 days post-challenge (pc) for clinical manifestations and mortality records.

Results

IBV isolation and purification

Regular IBV lesions were noticed in 13 embryo samples including curling and dwarfism with subcutaneous hemorrhages in embryos after successive five passages and embryo deaths were recorded in five samples only. Meanwhile, the embryo mortality at the first day post-inoculation was considered as non-specific deaths. Afterwards, the viral purification with RBCs washing technique showing negative results for presence of any haemagglutinating agents with the HA test after 4 sets of trials and was confirmed also with negative RT-PCR results for HA viruses including NDV and avian influenza viruses.

RT-PCR identification

The harvested allantoic fluid of the 30 samples were screened by RT-PCR and four samples only were showed positivity for IBV (2 from broiler flocks and 2 in layer flocks as in table.1) exhibiting the targeted band of the whole S1 gene (1760 bp) representing 13.3% of the collected samples as showed in figure.1.

S1 gene sequencing and phylogenetic analysis

The four positive RT-PCR samples in four different Egyptian governorates (Alexandria, El Behera, El Gharbia and Qalyubia) were subjected to Sanger dideoxy nucleotide sequencing and the resulted sequences were submitted to Genbank (Table.1).

The four study isolates IBV-CH-EG-QAL-VVT.N**RC-2021**, **IBV-CH-EG-ALEX-VVT.NRC-2021**, **IBV-CH-EG-GH-VVT.NRC.2021** and **IBV-CH-EG-BH-VVT.NRC-2021** were aligned and compared to other Egyptian IBV sequences and vaccine like strains available in Genbank in which, the 4 isolates found all clustered in GI.23 clade in compared to representative circulating Egyptian and israelian strains with closely relation to Egyptian variant II group. While found spaced out from vaccine strains like H120, 4/91, M41 and Ma5 strains (Figure.2).

Furthermore, table.2 shows the results of amino acid identity analysis, in which the results indicated that the study isolates have a high identity percent among each others found ranged from 98.2% to 99.1%. While their identity to other Egyptian variants like **IBV-Ck-EG-Fadllah-10-2019** and **Ck-Eg-BSU-2-2011,** showed high consistency varied from 97.9% to 98.6%. Moreover, the identity percent of our isolates compared to the Israelian strains **ISR-667-2021** and **IS-82-2020** revealed little higher similarity ranged from 96.0% to 96.6%. In addition, the amino acid identity of our isolates

compared to vaccine strains like 4/91, H120, Ma5 and Connecticut-46 strains were found of low homology ranged from 38.1% to 80.3%.

Isolates of the study.
Pathogenicity challenge trial

For assessment of pathogenicity analysis, the present study isolates were prepared for a challenge trial in 40 birds of four groups of 10 birds each. In challenged groups 1, 2, 3 and 4 the birds started exhibiting signs of ruffled feather and decreased food and water intake 3 days pc with the four IBV isolates. By the day 4 pc birds in infected groups develop respiratory signs, nasal discharges and rales whereas all challenged birds were depressed and morbidity reached 100% in all IBV challenged groups. At the day 5 to 7 pc the clinical picture was very apparent and mortality was recorded as (n= 3/10) in groups 1 and 3 while (n=4/10) in groups 2 and 4 giving mortality rates of 30% and 40% respectively. While in the control negative group 5 all birds were active and alert with no detected signs or mortality. In addition to, necropsy examination of dead birds showed severe nephritis with swollen kidneys and bloody caseated tracheitis with petichial hemorrhages in lung and cecal tonsils. On the other hand, no post-mortem gross lesions were observed in mock-challenged control group during the monitoring period pc.

Discussion

In Egypt, IB is considered one of the most devastating respiratory pathogen that impact the poultry industry as indicated by many surveillance results in the last recent years in spite of the aggravating vaccination schemes applied to poultry field [12, 13, 14, 15, 16]. Therefore, the goal of the present investigation is to isolate and characterize the recent currently epidemic strains of IBV in Egypt and comparing them with others previously isolated in Egypt and nearby countries.

In the current investigation, 30 samples were collected from kidneys and trachea pool from vaccinated chicken flocks suffering from respiratory signs accompanied with renal lesions. According to OIE (10), the embryonated SPF eggs inoculation is still considered a premium method for IBV diagnosis and isolation, whereas in our study the pathognomonic lesions of curling and dwarfing were recorded in the chicken embryos inoculated with the suspected IBV samples after five consecutive passages as recently described by Cook et al. (17).

The traditional diagnosis of IBV is depending on viral isolation in ECE followed by RT-PCR as a rapid technique for absolute detection of IBV strains. So the harvested allantoic fluid in our study was examined all by conventional RT-PCR and from out of 30 samples, 4 samples show positivity for IBV specific band of the whole S1 gene as previously mentioned by some authors (11, 18).

Further identification by genotyping, full-length S1 gene sequencing and phylogenetic analysis revealed that The 4 study isolates *IBV-CH-EG-QAL-VVT.NRC-2021, IBV-CH-EG-ALEX-VVT.NRC-2021, IBV-CH-EG-GH-VVT.NRC.2021* and *IBV-CH-EG-BH-VVT.NRC-2021* found all clustered in GI.23 clade in compared to representative circulating Egyptian and israelian strains with closely relation to Egyptian variant II group. While found spaced out from vaccine strains like H120, 4/91, M41 and Ma5 strains and also the study isolates have a high identity percent among each others found ranged from 98.2% to 99.1%. While their identity to other Egyptian variants showed high consistency reached 98.6%. In addition, the amino acid identity of our isolates compared to vaccine strains like 4/91, H120, Ma5 and Connecticut-46 strains were found of low homology ranged from 38.1% to 80.3% as come in agreement with previous studies by some researches [9, 19] who isolated IBV strains closely related to egyptian variants from vaccinated chicken flocks. Furthermore, Rohaim et al. and Yehia et al. (15, 20) were capable of identifying a new variant strains from IBV related to GI.23 clade Egyptian var II group. In addition to, Hammouda et al. (21) investigated that their study isolates of IBV were closely related to Egyptian variant II group with lowest amino acid identity to M41 vaccine strain. Moreover, the likeness between the Egyptian isolates and others from neighboring countries like Israel representing 96.0% to 96.6%, whereas this is may be due to uncontrolled movement of persons and contraband objects through borders (22, 23).

The pathogenicity analysis is a paramount tool in assessment of viral virulence, so as to the current study isolates *IBV-CH-EG-QAL-VVT.NRC-2021, IBV-CH-EG-ALEX-VVT.NRC-2021, IBV-CH-EG-GH-VVT.NRC.2021* and *IBV-CH-EG-BH-VVT.NRC-2021* were employed for a challenge trial in 25 days-old commercial broiler chickens, whereas the four variant IBV isolates were capable of inducing different clinical manifestations of respiratory signs, depression, off-food and nasal

discharges with variable levels of mortality and 100% morbidity 7 days pc, as well as the IBV isolates exhibited affinity for post-mortem lesions in kidneys with marked nephritis and kidneys swelling or even pneumonia in challenged birds. This is harmonized with previous investigations of some researchers (24**, 25)**, moreover**,** Zanaty et al. **[13]** demonstrated that the two variant subgroups (Egy/Var-I and Egy/Var-II) found to be highly virulent to SPF chicks with mortalities up to 50% than those of the classic group with low virulence (10% mortality) after experimental infection with IBV variant strains.

Conclusion

In the present investigation and during our epidemiological surveillance, the IBV variants clustered in GI.23 and related to Egy var II group is still circulated and endemic in Egypt causing persistent infection to chicken flocks in spite of extensive vaccination regimes. As well as, such variants are proved to be virulent and pathogenic to broiler chickens resulted in various mortalities and morbidity that complicating the poultry field situation in Egypt.

Author's Contribution

All authors equally participated in design, experimental procedure, writing, revised, and reviewing the manuscript.

Acknowledgments

The authors thank the laboratory of Veterinary Vaccines Technology (VVT), Central Labs, National Research Centre, Dokki, Cairo, Egypt for all kind of supports.

Conflict of interest

The authors have declared no conflict of interest.

Funding Statements

No funding was received for management of this study.

Fig. 1. Agarose gel electrophoresis pattern of the amplified products (1760 bp) by RT-PCR.

M: 1 kb DNA ladder; Lane 1: Control positive for IBV; Lane 2, 5, 6, 7 and 9: Negative IBV samples; Lane 3, 4, 8 and 10: Positive IBV specific bands of 1760 bp amplicon.

TABLE 1. Features of obtained Egyptian IBV isolates of the present study

Isolate name	Accession number	Locus	Genotype	Host	Isolation year	Province
IBV-CH-EG-QAL-VVT. NRC-2021	**OL415756**	**S1 gene**	**GI.23**	**Broilers**	**2021**	**Qalyubia**
IBV-CH-EG-GH-VVT. NRC.2021	**OL321756**	**S1 gene**	**GI.23**	**Broilers**	**2021**	**El Gharbia**
IBV-CH-EG-ALEX-VVT.NRC-2021	**OL321757**	**S1 gene**	**GI.23**	**Layers**	**2021**	**Alexandria**
IBV-CH-EG-BH-VVT. NRC-2021	**OL321755**	**S1 gene**	**GI.23**	**Layers**	**2021**	**El Behera**

TABLE 2. Amino acid sequence identity of obtained IBV isolates with other strains circulating in Egypt and vaccines showing identity and divergence percent, black squares indicate identical sequence.

Percent Identity

Divegence

	1	2	3	4	5	6	7	8	9	10	11	12		
1		98.9	98.2	99.1	98.3	98.4	96.4	96.1	80.3	77.3	78.5	38.5	1	▶IBV-CH-EG-ALEX-VVT.NRC-2021
2	1,1		98,9	99,0	98,6	97,9	96,2	96,5	80,0	76,3	77,6	38,4	2	▶IBV-CH-EG-BH-VVT.NRC-2021
3	1.8	1.1		99.1	97.9	98.1	96.0	96.6	79.1	80.1	79.9	38.1	3	▶IBV-CH-EG-GH-VVT.NRC.2021
4	0.9	1.0	0.9		98.0	98.3	96.5	96.6	77.1	78.0	77.6	40.9	4	▶IBV-CH-EG-QAL-VVT.NRC-2021
5	1.7	1.4	2.1	2.0		98.8	97.0	98.8	33.0	33.6	33.7	14.7	5	Ck-Eg-BSU-2-2011
6	1.6	2.1	1.9	2.7	1.2		97.3	96.7	92.3	82.9	82.3	39.2	6	IBV-Ck-EG-Fadllah-10-2019
7	3.6	3.8	4.0	3.5	3.0	2.7		91.3	38.2	39.2	39.0	18.8	7	IS-82-2020
8	3.9	3.5	3.4	3.3	1.2	3.3	7.0		41.9	41.8	41.8	20.4	8	ISR-667-2021
9	26.2	26.5	25.1	25.1	24.9	9.4	28.3	28.1		81.6	81.2	38.7	9	4-91
10	22.7	23.7	23.5	23.7	24.3	23.4	23.9	27.6	25.2		98.5	39.1	10	H120
11	21.5	22.4	23.9	24.2	23.6	24.4	24.6	27.8	25.9	1.8		38.7	11	MA5
12	123.2	123.4	121.1	125.1	107.5	122.1	124.9	125.2	125.0	121.3	124.6		12	Connecticut-46
	1	2	3	4	5	6	7	8	9	10	11	12		

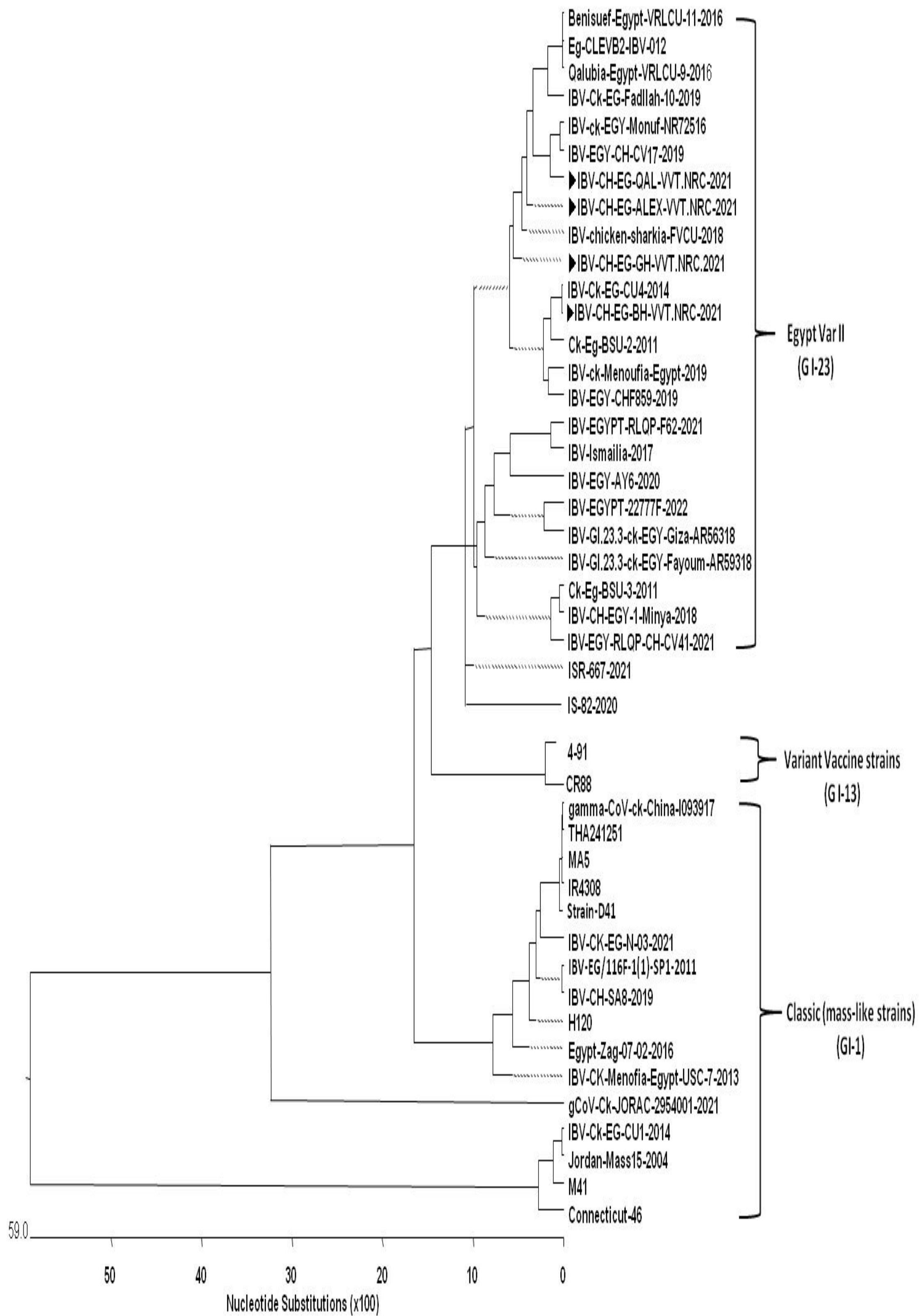

Fig. 2. Phylogenetic relationship between the IBV isolates obtained in this study and other previously isolated in Egypt with some reference and vaccinal strains retrieved from the Genbank by Maximum likelihood method.

References

1. King, A.M.Q., Adams, M.J., Carstens, E.B and Lefkowitz, A.J. Virus taxonomy: Ninth report of the International Committee on Taxonomy of Viruses. Elsevier, Oxford (2012).

2. Holmes, K.V and Lai, M.M. Coronaviridae: the virus and their replication. In: Fields BN, Knipe PM (eds) Fundamental virology, 4th edn. Lippincott-Raven, Philadelphia, pp 1163–1203 (2001).

3. De Wit, J.J.S and Cook, J.K.A. Spotlight on avian pathology: Infectious bronchitis virus. *Avian Pathol.*, **48**, 393–395 (2019).

4. Valastro, V., Holmes, E.C., Britton, P., Fusaro, A., Jackwood, M.W., Cattoli, G and Monne, I. S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. *Infect. Genet. Evo.,* **39**, 349–364 (2016).

5. Shan, D., Fang, S., Han, Z., Ai, H., Zhao,W., Chen, Y., Jiang, L. and Liu, S. Effects of hypervariable regions in spike protein on pathogenicity, tropism, and serotypes of infectious bronchitis virus. *Virus Res.*, **250**, 104–113 (2018).

6. Ahmed, H.N. Incidence and Treatment of Some Infectious Viral Respiratory Diseases of Poultry in Egypt. Ph.D. Thesis, Cairo University, Giza, Egypt (1954).

7. Taha, M., Moustafa, M.M., Mohamed, S., Attia, S.A. and Hatem, M.E. Effect of both mycoplasma galliseplicum and infectious bronchitis virus classical and variant strains on the egg production of broiler breeder flocks. *Vet. Med.*, **39**, 169–179 (1991).

8. Abdel-Moneim, A.S., Afifi, M.A. and El-Kady, M.F. Emergence of a novel genotype of avian infectious bronchitis virus in Egypt. *Arch. Virol.*, **157**, 2453–2457 (2012). https://doi. org/10.1007/s00705-012-1445-1.

9. Abozeid. H., Paldurai, A., Khattar, S.K., Afifi, M.A., El-Kady, M.F., El-Deeb, A.H. and Samal, S.K. Complete genome sequences of two avian infectious bronchitis viruses isolated in Egypt: Evidence for genetic drift and genetic recombination in the circulating viruses. *Infection. Genetics. and Evolution*, **53**, 7-14(2017) https://pubmed.ncbi.nlm.nih.gov/28495648/

10. OIE. Avian infectious bronchitis virus. OIE Terrestrial Manual. Scotto-Lavino, E., Du, G., Frohman, M.A., 2006. 3′ end cDNA amplification using classic RACE. *Nat. Protoc.***,** **1**, 2742–2745 (2013).

11. Feng, K.Y., Chen, T., Zhang, X., Shao, G.M., Cao, Y., Chen, D.K., Lin, W.C., Chen, F. and Xie, Q.M. Molecular characteristic and pathogenicity analysis of a virulent recombinant avian infectious bronchitis virus isolated in China. *Poult. Sci.,* **97**,3519–3531 (2018).

12. Zanaty, A., Arafa, A., Hagag, N. and El-Kady, M. Genotyping and pathotyping of diversified strains of infectious bronchitis viruses circulating in Egypt. *World J. Virol.*, **5**(3), 125-134 (2016a).

13. Zanaty, A., Naguib, M.M., El-Husseiny, M.H., Mady, W., Hagag, N. and Arafa, A. The sequence of the full spike S1 glycoprotein of infectious bronchitis virus circulating in Egypt reveals evidence of intra-genotypic recombination. *Arch. Virol.*, **161**,3583–3587 (2016b) .DOI 10.1007/s00705-016-3042-1

14. Abozeid, H.H. and Naguib, M.M. Infectious bronchitis virus in Egypt: Genetic diversity and vaccination strategies. *Veterinary Sciences,* **7**(4), 204 (2020**)**. https://DOI.org/10.3390/vetsci7040204

15. Rohaim, M.A., El Naggar, R.F., Helal, A.M., Bayoumi, M.M., El-Saied, M.A., Ahmed, K.A., Shabbir, M.Z. and Munir, M. Genetic diversity and phylodynamics of avian coronaviruses in egyptian wild birds. *Viruses*, **11** (1), 57 (2019a) https://doi.org/10.3390/

16. Rohaim, M.A., El Naggar, R.F., Hamoud, M.M., Bazid, A.I., Gamal, A.M., Laban, S.E., Abdel-Sabour, M.A., Nasr, S.A.E., Zaki, M.M., Shabbir, M.Z., Zahran, O.K. and Munir, M. Emergence and genetic analysis of variant pathogenic 4/91 (serotype 793/B) infectious bronchitis virus in Egypt during 2019. *Virus Genes*, **55**, 720–725(2019b) https:// doi.org/10.1007/s11262-019-01693-9.

17. Cook J.K., Jackwood, M. and Jones, R.C. The long view: 40 years of infectious bronchitis research. *Avian Pathol.*, **41**, 239-250 (2012) http://dx.doi.org/10.1080/03079457.2012.680432

18. Samir, M., Selim, A., Arafa, A., Khaleil, S., Hagag, N., Samir, A. and Hassan, M. Molecular Diversity between Field Isolates and Vaccinal Strains of Avian Infectious Bronchitis Virus in Egypt. *Global Veterinaria*, **13** (5), 820-827 (2014).

19. Abdel-Sabour, M.A., Al-Ebshahy, E.M., Khaliel, S.A., Abdel-Wanis, N.A. and Yanai, T. Isolation and Molecular Characterization of Novel Infectious Bronchitis Virus Variants from Vaccinated Broiler Flocks in Egypt. *Avian Diseases,* **61**,307–310 (2017).

20. Yehia, N., Said, D. and Zanaty, A.M. Characterization and Analysis of the Major Structural Protein Genes of the Recently Isolated Avian Infectious Bronchitis Virus in Egypt. *J. World Poult. Res.,* **10** (4), 649-661(2020) https://dx.doi.org/10.36380/jwpr.2020.74.

21. Hammouda, M.M., Arafa, A.M. and Madbouly, H.M. Molecular characterization and genotyping of recent isolate of Infectious Bronchitis Virus (IBV) in Egypt. *Journal of Veterinary Medical Research* , **26** (1), 115-124 (2019).

22. Mahmood, Z.H., Sleman, R.R. and Uthman, A.U. Isolation and molecular characterization of Sul/01/09avian infectious bronchitis virus, indicates the emergence of a new genotype in the Middle East. *Vet. Microbiol*., **150**,21–27 (2011).

23. Kasem, S., Abdel-Kader, A., Tahoon, A. and Shaban, H. Phylogenetic analysis of recent infectious bronchitis virus isolates from broiler chicken farms in Kafrelsheikh, Egypt. *Benha Veterinary Medical Journal*, **29** (1),189-195 (2015).

24. Sun, C., Han, Z., Ma, H., Zhang, Q., Yan, B., Shao, Y., Xu, J., Kong, X. and Liu, S. Phylogenetic analysis of infectious bronchitis coronaviruses newly isolated in China, and pathogenicity and evaluation of protection induced by Massachusetts serotype H120 vaccine against QX-like strains, *Avian Pathology*, **40**(1), 43-54 (2011). DOI: 10.1080/03079457.2010.538037

25. YuDong, W., Yong-Lin, W., ZiChun, Z., GenChe, F., YiHai, J. and Xiang, L. Isolation and identification of glandular stomach type IBV (QX IBV) in chickens. *Chinese Journal of Animal Quarantine*, **15**, 1-3 (1998).

التنميط الجينى الجزيئى ودراسة مرضية عن فيروس الإلتهاب الشعبى المعدى والمتفشى حاليا فى قطعان الدجاج بشمال مصر خلال عام 2021

سامح عبد المعز عامر ، هاجر مجدى أحمد و أسماء محمود معتوق
قسم أمراض الدواجن - معهد البحوث البيطرية - المركز القومى للبحوث - القاهرة - مصر الرقم البريدى 12622

أصبح فيروس التهاب الشعب الهوائية المعدي (IBV) متوطنًا في مصر مع حدوث جائحات مرضية متعددة منه في كل من دجاج التسمين اللاحم والدجاج البياض على الرغم من برامج التحصين المكثفة.إن الهدف من الدراسة الحالية هو تحديد الخصائص الوراثية والمرضية لفيروسات التهاب الشعب الهوائية المعدي المنتشرة حاليًا في مختلف المحافظات المصرية. تم فحص ثلاثين قطيع من الدجاج لها تاريخ تطعيم ضد فيروس التهاب الشعب الهوائية المعدي باستخدام تكاثر البيض وإختبار البلمرة الحلقى المتسلسل العكسى (RT-PCR) التي تستهدف جين البروتين (S) كما تم إجراء تحليل تسلسل الجين S1 كاملا. بالإضافة إلى ذلك، تم تقييم السمات المرضية لعزلات فيروس التهاب الشعب الهوائية المعدي التي تم الحصول عليها من خلال إختبار التحدي في دجاج التسمين التجاري بعمر 25 يومًا. كشفت النتائج أن عزلات فيروس التهاب الشعب الهوائية المعدي الأربعة الإيجابية التي تم الحصول عليها هي IBV-CH-EG-QAL-VVT.NRC-2021)، IBV-CH-EG-ALEX-VVT. NRC-2021، IBV-CH-EG-GH-VVT. 2021, NRC و (IBV-CH-EG-BH-VVT.NRC-2021 تقع في سلالة النمط الوراثي المتميز ((GI.23 ومن الناحية التطورية مرتبطة بمجموعة المتغير المصري ما يسمى بالمتغير المصري 2 (Egy/Var2) المتعلقة بآخرين سبق تسجيلهم في مصر والدول المجاورة. علاوة على ذلك، وجد أن العزلات الأربع ممرضة لدجاج التسمين بنسبة نفوق مختلفة 30 و40% بالإضافة إلى وجود آفات مرضية وتشريحية فى الجهاز التنفسي والكلى. أشارت هذه النتائج إلى انتشار سلالات فيروس التهاب الشعب الهوائية المعدي في قطعان الدجاج في مصر ذات السمات شديدة الإمراض والتي تضرب صناعة الدواجن بشدة وتتسبب في خسائر اقتصادية فادحة حتى في القطعان المحصنة.

الكلمات الدالة: فيروس الالتهاب الرئوي المعدى-التصنيف الجيني-الممرض