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RESEARCH ARTICLE

Phylogenetic Analysis of Infectious Bronchitis Viruses Currently Circulating in the Egyptian Field

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Abstract

One of the major problems of infectious bronchitis virus (IBV) is the frequent emergence of new variants in the Egyptian field. In the present study, 42 broiler chicken flocks suffered from respiratory troubles were investigated for infectious bronchitis virus in 10 Governorates in Egypt during 2016-2018. Ten out of 42 examined flocks revealed variable degrees of embryo lesions after 3-5 passages with negative heamagglutination (HA) activity. From these 10 flocks, IBV was confirmed in six flocks using reverse transcriptase polymerase chain reaction (RT-PCR) assay. Four IBV-positive isolates were selected for further sequence analysis. Partial sequencing of S1 gene revealed four IBV variant-2 isolates circulating among chickens in Egypt. These isolates are IB-Beh-Ch-F2-2016, IB-Sh-Ch-F25-2017, IB-Sh-Ch-F41-2018 and IB-Sh-Ch-F42-2018 and submitted on Gen Bank with accession numbers MH460643, MH460644, MK408615 and MK408616, respectively. The IB-Sh-Ch-F25-2017 isolate had only one amino acid substitution while IB-Beh-Ch-F2-2016, IB-Sh-Ch-F41-2018 and IB-Sh-Ch-F42-2018 isolates had much higher genetic diversity. The similarity between classic viruses of vaccine origin used in Egypt (H120, Ma5) and our four filed isolates ranged between 75.7 and 80.4%. It could be concluded that IB variant-2 strains still circulate in the Egyptian field in spite of vaccination. Therefore we need to revise the IBV vaccines used in Egypt and try to prepare local vaccines with periodic evaluation of cross protection of such vaccines.

Key words: IBV- Variant 2, RT-PCR, Broilers, Genetic diversity, Egypt.

Introduction

Avian infectious bronchitis (IB) is one of the major infectious diseases affecting poultry industry worldwide. This disease usually occurs in both vaccinated and non-vaccinated chickens causing severe economic losses due to mortality, decreased productivity and control and prevention costs [1, 2]. The IB disease is caused by infectious bronchitis virus (IBV) belongs to the genus *Coronavirus* in the family *Coronaviridae.*

Infectious bronchitis virus is an enveloped, positive-sense, single stranded RNA genome with approximately 27 kb in length. The virion has four structural proteins, namely the nucleocapsid (N) protein, membrane (M) protein, envelope protein (E) and spike (S) glycoprotein. The S glycoprotein is post-translationally cleaved into the S1 and S2 subunits [3]. Neutralizing and serotype-specific epitopes are associated with the defined hyper variable region (HVR) in the S1 subunit, therefore it`s important to perform molecular characterization of IBV based on analysis of the S1 gene [4, 5].

Diversity in S1 gene probably results from nucleotide point mutations, insertions, deletions or RNA recombination of the S1 genes [6, 7] or the use of multiple vaccines, which cause immune pressure that might lead to emergence of new variants [8]. Consequently, the evolution of new IBV strains is due to simultaneous infection of multiple virus types and the use of live vaccines makes vaccination is only partially successful due to continual emergence of antigenic variants [9].

Many variant strains have been reported previously from different Governorates in Egypt such as Egypt/ Beni-Seuf/01 [10], EgCLEVB-

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1IBV012 and EgCLEVB-2IBV012 [11], Eg/1265B/2012 and Eg/12120s/2012 [12] and IBV-KFS 1, IBV-KFS 2 and IBV-KFS 3 [13]. The basic problem of IBV is vaccination failure, which is mainly due to the frequent emergence of new variants that differ antigenically from vaccine serotypes [14, 15]. Moreover, Identification of these variant serotypes circulating in the Egyptian field is very important for screening the new variants as well as selecting the most appropriate vaccine strains [16]. Additionally, limited information is available about genetic diversity of IBV strains circulating among poultry in Egypt. Therefore, the aim of our study was to determine genetic characterization of IBV field isolates circulating in different governorates in Egypt during the period from 2016-2018.

Material and Methods

Birds and Sample collection

A total of 168 birds with respiratory troubles representing 42 commercial broiler chicken flocks (4 birds/each flock) were collected during the period of 2016-2018. These examined flocks were located in ten different Governorates in Egypt: Sharkia (n=18), Damietta (n=7), Alexandria (n=1), Dakahlia (n=2), kaliobia (n=2), Gharbia (n=1), Monufia (n=4), Ismailia (n=1), Sinai (n=1) and Behera (n=5). Descriptive data of theses flocks were illustrated in Table 1.Clinical and postmortem examinations were recorded. Forty-two tissue pools (4 birds/pool/each flock) of respiratory organs (trachea, bronchi and lung) were used for IB virus isolation trials. The collected samples were stored at -20^{0}C till used. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University.

Table 1: The Descriptive data of the investigated broiler chicken flocks for detection of infectious bronchitis virus during 2016-2018 in some Governorates in Egypt.

Flock number	Date	Locality	No. of birds	Age/ day	Breed	Previous vaccination	Mortality*
1	2016	Damietta	10000	30	Sasso	Not Vaccinated	6.7%
2	2016	Beheira	5000	22	Ross	Hitchner+ IB (7d)	10.4%
3	2016	Gharbia	14000	24	Sasso	Hitchner+ IB(7d)	6.57%
4	2017	Damietta	9000	25	Sasso	Not Vaccinated	9.66%
5	2017	Monufia	19000	28	Cubb	Clone +IB(10d)	2.89%
6	2016	Sharkia	7000	30	Ross	Not Vaccinated	7.86%
7	2017	Beheira	20000	24	Cubb	Hitchner + IB(10d)	5.5%
8	2017	Sharkia	5000	29	Cubb	Not Vaccinated	9.6%
9	2017	Beheira	10000	30	Cubb	Hitchner+ IB(10d)	9.4%
10	2017	Sharkia	14000	31	Ross	Not Vaccinated	10.36%
11	2017	Beheira	10000	28	Cubb	Not Vaccinated	4.2%
12	2017	Sharkia	7000	27	Ross	Not Vaccinated	7.4%
13	2017	Damietta	7000	28	Sasso	Not Vaccinated	14.6%
14	2017	Beheira	3000	23	Cubb	Not Vaccinated	30%
15	2017	Alexandria	50000	25	Cubb	Not Vaccinated	1.34%
16	2017	Sharkia	4000	31	Cubb	Not Vaccinated	16.75%
17	2017	Ismailia	3000	24	Sasso	Hitchner + IB(4d)	28%
18	2017	Sharkia	13000	24	Ross	Not Vaccinated	6.46%
19	2017	Damietta	6000	28	Sasso	Not Vaccinated	9.17%
20	2017	Monufia	15000	25	Ross	Hitchner+ IB(4d)	15%
21	2017	Monufia	50000	29	Ross	Not Vaccinated	3.3%
22	2017	Damietta	6000	27	Ross	Not Vaccinated	11.67%
23	2017	Sharkia	10000	22	Ross	Not Vaccinated	17.6%
24	2017	Sharkia	8000	25	Sasso	Not Vaccinated	14.37%
25	2017	Sharkia	5000	29	Cubb	Not Vaccinated	13.4%
26	2017	Sharkia	5000	23	Cubb	Clone +IB(9d)	15%
27	2017	Qalyubia	8000	27	Cubb	Not Vaccinated	6.5%
28	2017	Dakahlia	7000	22	Ross	Not Vaccinated	9.14%
29	2017	Sharkia	3000	28	Cubb	Not Vaccinated	12.33%
30	2017	Sinai	6000	27	Cubb	Not Vaccinated	6.17%

Table 1: Continued

Flock	Date	Locality	No. of	Age/	Breed	Previous vaccination	Mortality*
31	2017	Damietta	10000	32	Cubb	Not Vaccinated	6.8%
32	2017	Sharkia	5000	25	Cubb	Hitchner +IB (7d)	11.5%
33	2017	Qalyubia	6000	27	Cubb	Not Vaccinated	8%
34	2017	Dakahlia	14000	25	Sasso	Not Vaccinated	8.28%
35	2017	Damietta	10000	28	Sasso	Not Vaccinated	7.4%
36	2017	Monufia	6000	27	Cubb	Not Vaccinated	8%
37	2017	Sharkia	9000	25	Cubb	Not Vaccinated	12.89%
38	2017	Sharkia	6000	27	Cubb	Not Vaccinated	8.67%
39	2017	Sharkia	10000	25	Cubb	Not Vaccinated	11.5%
40	2017	Sharkia	14000	25	Cubb	Not Vaccinated	6.14%
41	2018	Sharkia	5000	25	Cubb	Clone + IB(7d)	15%
42	2018	Sharkia	7000	65	Sasso	Not Vaccinated	1.77%

* Mortality rates within 3 days

Sample preparation and virus isolation

Suspensions of pooled respiratory tissues were prepared in sterile phosphate buffered saline (10% w/v) with addition of penicillin G sodium (10000 I.U/mL) and streptomycin (1mg/mL). The suspensions are clarified by low-speed centrifugation and filtration through bacteriological filters (0.2 µ). Five embryonated chicken eggs (ECE) from apparently healthy, non - IBV vaccinated commercial breeders (Abo ammar, El-masalmia, Egypt), 10 days old were inoculated for each pooled sample (one pooled sample/each flock). The embryos were inoculated with 0.2 mL of the sample into the allantoic cavity then incubated at 37 °C and candled daily to check for embryonic viability. Un-inoculated five ECE were included as negative control. Any early deaths within 24 h post-inoculations were excluded due to non-specific death. All samples were blindly passaged for (3-5) times till recording of the embryonic lesions. All embryos were collected and left to be chilled at 4ºC overnight then examined for IBV characteristic lesions. Moreover, the allantoic fluids were harvested aseptically and examined for HA activity [17].

Molecular characterization of HVR-3 of S1 gene

The harvested allantoic fluids from 10 suspected samples (samples that gave variable degrees of the characteristic IBV embryo lesions and -ve HA) were further subjected to the amplifications of HVR-3 of the S1 gene using Qiagen one-step RT-PCR Kit (Qiagen, GmbH, Hilden, Germany). The RT-PCR was carried out in Biometra T thermal cycler machine (biometra-Germany) using IBV-HVR3- FW 5' TAC TGG TAA TTT TTC AGA TGG '3 and IBV-HVR3-RV 5' CAG AYT GCT TRC AAC CAC C '3 [18]. The cycling condition was 45°C for 10 min then an initial denaturation step at 95 °C for 10 min followed by 35 PCR amplification cycles were run at 95°C for 15 s, 52°C for 15 s and 68°C for 30 s with a final extension step of 68°C for 5 min. The expected PCR product size is 382 bp. The amplicons were purified using the QIA quick gel extraction kit (Qiagen, GmbH, and Hilden, Germany) according to the manufacturer's instructions. The purified PCR products of four selected samples were sequenced using Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA) and Applied Biosystems 3130 automated DNA Sequencer (ABI, USA).

Sequence analysis

The HVR3-S1 gene sequences of field isolates included in this study were compared with previously published IBV reference and vaccine strains available in the public database (NCBI, United States) (Table 2). A phylogenetic tree of the nucleotide and amino acid sequences was constructed using MEGA version 6 software **[**19]. A comparative analysis of deduced amino acids and nucleotide sequences of the HVR-3 was created using the CLUSTAL W Multiple Sequence Alignment Program, version 1.83 of MegAlign module of Lasergene DNAStar software.

Table 2: Reference and vaccine IBV strains included in comparative sequence analysis of S1 gene.

Virus ID	Accession #	Origin	Year	Genotype
IBV-Eg-12120s-2012	KC533684	Egypt	2012	Var-2
IBV-EGY-F-03	DQ487085	Egypt	2003	Classic
IBV-Ma5	KU736747	Brazil	2013	Classic-vaccine
IBV-Connecticut	EU283061	USA	2016	Classic- vaccine
IBV-M41	FJ904720	USA	1965	Classic
IBV-D274	MH021175	Netherlands	1979	variant-1
IBV-QXIBV	AF193423	China	1999	Variant
IBV-UK-4-91	JN192154	UK	2011	variant-1
IBV-VAR2-06	JX027070	Israel	2006	variant-2
IBV-attenuated-IS-1494-06	HM131453	Israel	2006	variant-2
IBV-Israel-720-99	AY091552	Israel	1999	variant-1
IBV-IS-885	AY279533	Israel	2000	variant-2
IBV-isolate-variant-2	AF093796	Israel	2001	variant-2
IBV-strain-D207	M21969	Netherlands	1989	variant-1
IBV-CK-CH-LDL-01I	DQ167130	China	2001	variant-1
IBV-isolate-J2	AF286303	Singapore	2000	Variant
IBV-strain-J9	DQ515802	China	2003	Variant-vaccine
IBV-strain-H120	KU736750	Brazil	2013	Classic-vaccine
IBV-strain-CR88121	JN542567	France	1998	variant-1
IBV-isolate-variant-1	AF093795	Israel	1999	variant-1

Results

Clinical and Postmortem findings

Clinically examined birds showed general signs of illness in addition to respiratory signs in the form of sneezing, coughing, nasal discharges, conjunctivitis, rales and gasping in all flocks (Figure 1A,B). Postmortem examination of both freshly dead and sacrificed diseased birds revealed caseated plugs at tracheal bifurcation (no=42) (Figure 1C). Additionally, moderate pneumonia and cloudiness of air sacs with or without yellow caseous exudates were also observed (Figure 1D).

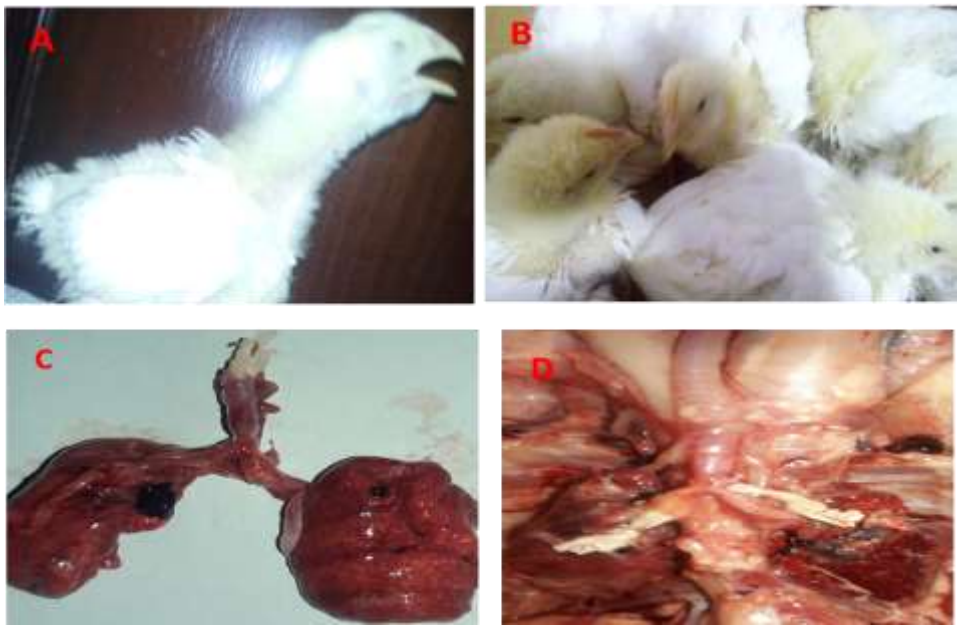

Figure 1: Clinical and Postmortem findings of the investigated chicken flocks. (A) Broiler chicken of 25 days old (flock No. 41) showing respiratory manifestations including gasping. (B) Broiler chickens of 22 days old (flock No.2) showing general signs of illness (depression and huddling together). (C) Trachea of 23 days old broiler chicken (farm No.14) containing caseated plug in tracheal bifurcation. (D) Trachea of 24 days old Sasso chicken (flock No.17) containing caseated plug in tracheal bifurcation and Cloudiness of air sacs.

ECE inoculation and HA activity

Ten out of 42 examined pooled samples revealed variable degrees of curling and dwarfing of the inoculated embryos after 3-5 passages with negative HA activity. The other 32 ones revealed embryo death within 72 hrs post inoculation with positive HA activity. The relatedness of clinical observations and virus isolation results was illustrated in Figure 2A.

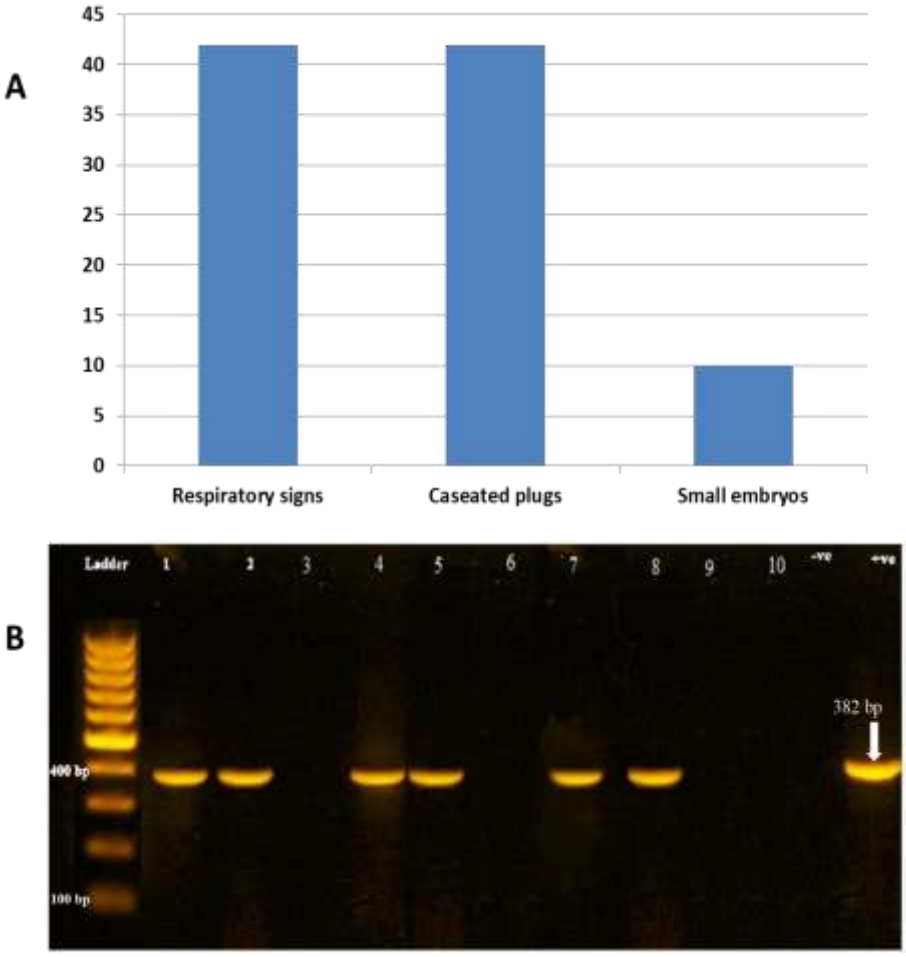

Figure 2: (A) Relationship between field and laboratory diagnosis of IBV. (B) Agarose gel electrophoresis of RT-PCR amplicons of IBV. Lanes 1, 2, 4, 5, 7and 8: positive IBV samples represent the flock No. 2, 12, 19, 25, 41 and 42. All positive samples showed band at 382 bp. Lanes 3, 6, 9 and 10: negative IBV samples represent the flock No. 4,9,21 and 27. Lane -ve: Negative control. Lane +ve: Positive control (H120 vaccine).

RT-PCR and sequencing

Amplification result of HVR3-S1 gene in 10 examined pooled samples (which gave variable degrees of embryo lesions on ECE and -ve HA activity) showed that 6/10 were positive to IBV and showed the specific expected bands at 382 bp (Figure 2B). Since the current samples were collected during the period of 2016-2018; four positive field isolates (representative for the three years of investigation) were selected for further sequence analysis. These four isolates are (IB-Beh-Ch-F2-2016, IB-Sh-Ch-F25-2017, IB-Sh-Ch-F41-2018 and IB-Sh-Ch-F42-2018) and submitted on Gen Bank with accession numbers MH460643, MH460644, MK408615 and MK408616, respectively.

Phylogenetic analysis

Phylogenetic tree showed that all investigated isolates were IBV variant - 2 as illustrated in Figure 3. IB-Beh-Ch-F2-2016 and IB-Sh-Ch-F25-2017 isolates belonged to other Egyptian variant - 2 strains like Eg-12120s-2012 strain. Meanwhile, IB-Sh-Ch-F41-2018 and IB-Sh-Ch-F42-2018 isolates had a close relationship with Israeli IBV variant- 2 like IBV/IS/885 and IBV/Israel/720/99 strains.

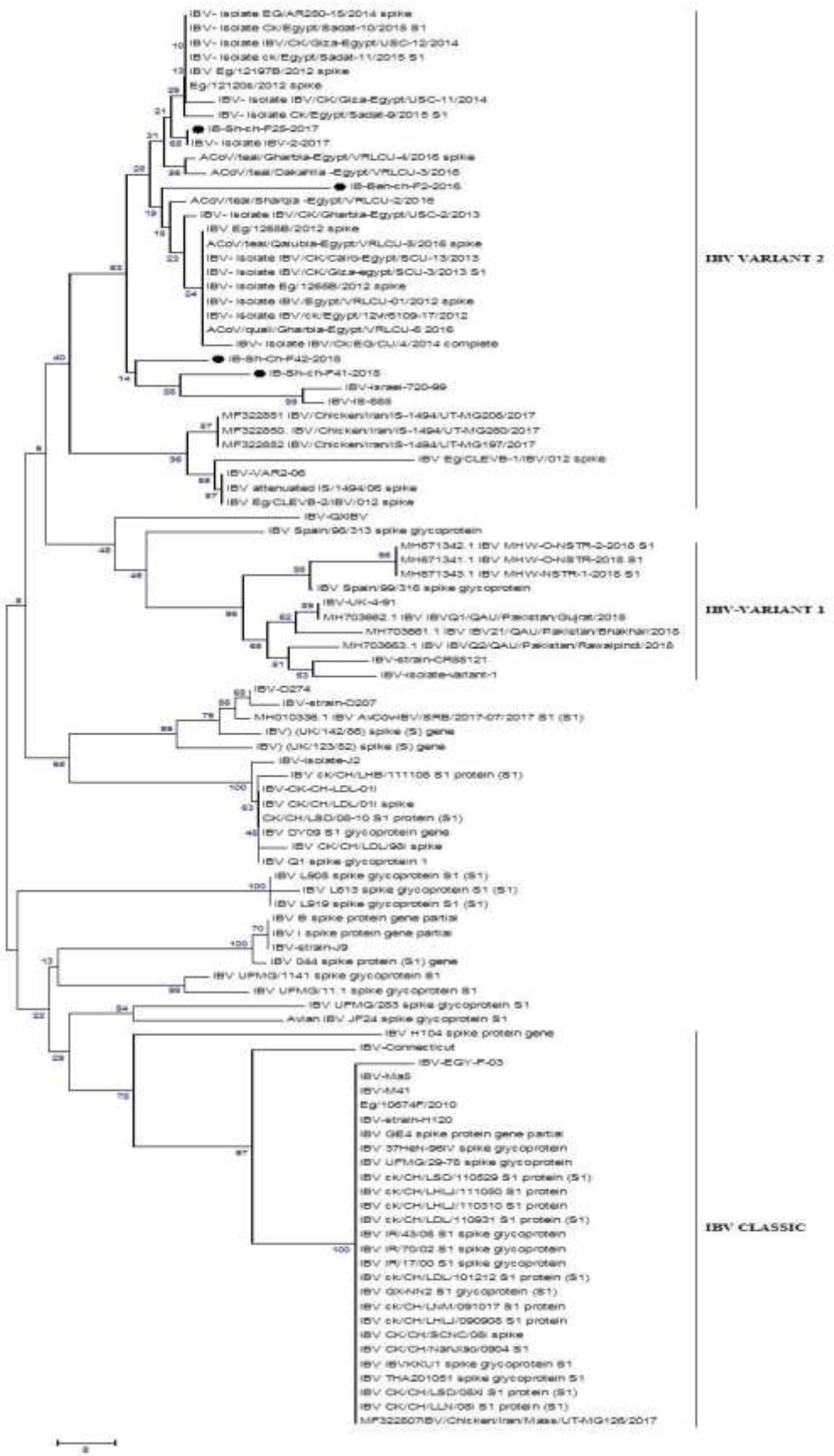

Figure 3: Phylogenetic tree pattern of the nucleotide sequence alignment of IBV isolates (IB-Beh-Ch-F2-2016, IB-Sh-Ch-F25-2017, IB-Sh-Ch-F41-2018, IB-Sh-Ch-F42-2018). The black dots refer to our field isolates. The figure was generated by MEGA 6 software.

Nucleotide and amino acid identity

The similarity of these field isolates with each other ranged from 90.7 to 95.3%. Meanwhile, it was close to the previous Egyptian virus (Eg-12120s-2012) of the same group and ranged from 92.5% to 94.4%. On the other hand, this similarity was reduced in comparison to variant -2 viruses of Israeli origin (like IBV-IS- 885 and IS/1494/06) and it was 81.3% to 91.6%. Interestingly, the lowest similarity of our field isolates was observed by comparing them with both classic viruses of vaccine origin (like H120 and Ma5) and the old variant-1 strains (75.7 to 80.4%).

These selected Egyptian isolates showed multiple silent amino acid mutations in HVR 3 of S1 gene when compared to the old Egyptian variant virus (IBV-Eg-12120s-2012). IB-Sh-Ch-F25-2017 isolate had only one amino acid substitution (H283Y) while IB-Beh-Ch-F2-2016, IB-Sh-Ch-F41-2018 and IB-Sh-Ch-F42-2018 isolates had much higher genetic diversity. Where IB-Beh-Ch-F2-2016 had 7 amino acid substitutions (V257L, R261H, T272N, H277Y, G303D, S313R and S314R). Meanwhile, there were 6 (H277Y, H283Y, L294F, F309L, S314G and P330k) and 5 (N263T, H277Y, H283Y, H298Q and P330K) amino acid substitutions in case of IB-Sh-Ch-F41-2018 and IB-Sh-Ch-F42-2018, respectively.

Discussion

Infectious bronchitis (IB) has still occurred extremely in both vaccinated and non-vaccinated chicken flocks causing severe economic losses to poultry industry in Egypt. Therefore, the objective of this study was to survey the Egyptian broiler chicken flocks for infectious bronchitis virus in Sharkia, Damietta, Dakahlia, Behera, Alexandria, Monufia, Sinia, Ismalia, Gharbia and Qalubia Governorates, Egypt and to study the genetic evolutions of the circulating infectious bronchitis virus (IBV) strains seeking the new variants emerged in the Egyptian field.

The obtained results revealed that the observed clinical findings among examined broiler chickens were depression, huddling together with decrease in feed intake. The respiratory troubles were the main and ranged from mild to remarkable sneezing, coughing, nasal discharges and gasping. The obtained findings were going in parallel with that previously recorded clinical signs [11, 20, 21].

The postmortem lesions included caseated plugs at tracheal bifurcation in all examined flocks. Additionally, small areas of pneumonia and cloudiness of air sacs with or without yellow caseous exudates were observed. Similar findings were reported previously in IBV single and mixed infections in chickens [11, 21]. The presence of cloudiness of air sacs with or without yellow caseous exudates indicated complication with bacterial infection. Similar finding was reported previously by Awad *et al*. [23] who recorded that IBV infection is commonly followed by secondary bacterial infection.

Results of IBV isolation in ECE revealed that, 10 flocks (2, 4, 9, 12, 19, 21, 25, 27, 41 and 42) showed variable degrees of curling and dwarfing of the inoculated embryos after 3-5 passages. The recorded results were in agreement with IBV isolation as recorded previously [21, 22]. Where, they reported that IBV caused curling, dwarfing and subcutaneous hemorrhages in inoculated embryos after several passages.

Embryo death within 72 hrs PI was recorded in 76.19% of inoculated samples. The allantoic fluids of these embryos revealed positive HA activity. This can be explained by the presence of virulent hemagglutinating viral infection that may be ND and /or AI viruses. Similar results were reported previously in a recent study conducted in Egypt [21] who recorded the rapid embryo death with positive HA activity in both ND and AI virus infections in Egyptian chicken flocks. Also another study in Egypt recorded the occurrence of HA viruses in 86% of the investigated flocks with respiratory problems in north Shrakia during 2012-2013 [24].

The concurrence of field findings and the laboratory IBV isolation was studied. It was found that 42 broiler chicken flocks suffering from respiratory troubles and have caseated plugs in tracheal bifurcation. Only 10 IBV isolates (represented to 10 examined flocks) were isolated from the respiratory tissues. The obtained findings could be explained by either

the high incidence of concurrent HA viruses detected in allantoic fluids of 32 samples or the presence of latent virus infection and /or maternal derived antibodies (MDA) in chicken embryos which may interfere with IBV replication [24,25].

The suspected IBV samples (10 samples/10 flocks) were subjected to amplification of S1 gene using specific primers. Six samples (2, 12, 19, 25, 41 and 42) showed expected band at 382 bp. Accordingly, the IBV was evidenced positive using the same primer band at 382 bp by Naguib *et al*. [18] who stated that the RT-PCR assay using a specific primer succeeded in detection of infectious bronchitis virus.

Out of these 6 positive isolates, 4 isolates (66.66%) were from non-vaccinated flocks (flock No. 12, 19, 25 and 42) and the other 2 isolates (33.33%) were from vaccinated ones (flock No. 2 and 41). These vaccinated flocks were vaccinated with classic virus vaccine. The obtained information indicates that IBV was isolated from both vaccinated and non-vaccinated flocks. This could be explained by little or no cross protection of classic vaccinal strains against field circulating IB viruses [11]. Similar results were reported early by Noussieur [22] who isolated IBV with percentages of 63 and 23% among non-vaccinated and vaccinated chicken flocks, respectively.

Results of phylogenetic analysis revealed that the four selected isolates present in the same group with Eg/1265B/2012, Eg/12120s/2012 [12] and EgCLEVB-1IBV012, EgCLEVB-2IBV012 [11] which are variant 2- strains. Similar results were reported previously by Nossieur [22] who sequenced 18 isolates and grouped all of them in EGY Variant II. Meanwhile, this result disagreed with those of Selim *et al.* [15] whom sequenced 11 IBV isolates and grouped all of them to Variant I.

The genetic analysis revealed that these field isolates were similar to each other (90.7 to 95.3% identity). Additionally, they showed 92.5 to 94.4% identity to the previous Egyptian isolate Eg-12120S-2012. The high antigenic identity between these isolates among wide geographic areas could refer to high contagious ability of the virus as well as absence or lack of biosecurity measures [26].

The similarity between the classic viruses of vaccine origin used in Egypt (H120, Ma5) and our 4 variant 2 isolates ranged between 75.7 and 80.4%. Nearly similar results were reported by Nossieur [22] who recorded 79.1, 79.1 and 78% identity to Ma5, H120 and M41, respectively. These results confirm that our field isolates are antigenically different from vaccinal strains supporting the break of vaccination and emerging of new viruses circulating in the Egyptian field among poultry flocks.

The present result revealed multiple silent mutations at different positions in the S1 gene in comparison to the old Egyptian viruses. These results agree with those obtained by Abdel Moneim *et al.*, [27] who reported that new IBV genotypes emerged as a result of few changes in the amino acid structure of the S1 protein. These new strains are emerged as a result of mutation, insertion or recombination [28].

Conclusion

It could be concluded that; IBV variant-2 strains is still circulating in the Egyptian field accompanied with HA viruses leading to bad economic impact on poultry industry in Egypt. These strains are antigenically different from vaccinal strains that emphasize the importance of continued monitoring of IBV for developing vaccines having strains genetically closely related to the circulated field viruses.

Conflict of Interest

None of the authors have any conflict of interest to declare

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الملخص العربي

التحليل الوراثي لفيروسات التهاب الشعب الهوائية المعدي المنتشرة حاليًا في الحقل المصري

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واحدة من المشاكل الرئيسية لفيروس التهاب الشعب الهوائية المعدي (IBV) هي الظهور المتكرر لعترات جديدة في الحقل المصري. في هذه الدراسة، تم دراسة ٤٢ قطيعًا من دجاج التسمين الذي يعاني من مشاكل تنفسية في ١٠ محافظات في مصر خلال الفترة ٢٠١٦-٢٠١٨. ١٠عينات من ٤٢ عينه (٢٣.٨ ٪) أظهروا درجات متفاوته من الاعراض الخاصة بالفيروس علي حجم الاجنه المحقونة بعد ٣-٥ تمريرات مع سلبية ظاهرة التلزن الدموى (HA). ثم تم التاكد من ستة معزولات (٦٠٪) باستخدام اختبار تفاعل انزيم البلمرة المتسلسل العكسي (RT-PCR). تم اختيار أربع معزولات ايجابيه من الفيروس لمزيد من التحليل التسلسلي الجيني. كشف التسلسل الجزئي لجين S1 عن أربعة معزولات من النوع المختلف variant 2 المتداولة بين الدجاج في مصر. هذه المعزولات الأربع هي (IB-Beh-Ch-F2-2016، IB-Sh-Ch-F25-2017، IB-Sh-Ch-F41-2018 , IB-Sh-Ch-F42-2018) وتم اضافتهم على بنك الجينات بالأرقام التالية MH460643 ، MH460644، MK40861 , MK408616 على التوالي. أظهرت المعزولة IB-Sh-Ch-F25- 2017تغير في حمض اميني واحد فقط في حين كانت المعزولات IB-Beh-Ch-F2-2016 , IB-Sh-Ch-F41-2018 , IB-Sh-Ch-F42-2018 أعلى بكثير في التنوع الجيني. و قد لوحظ أقل تشابه مع الفيروسات الكلاسيكية المستخدمة في التحصين (مثل H120 و Ma5) وكان يتراوح من ٧٥.٧ إلى ٨٠.٤ ٪. يمكن أن نستنتج أن عترات فيروس التهاب الشعب الهوائية المعدي من النوع المختلف variant - 2 لا تزال تنتشر في الحقل المصري على الرغم من التحصين. لذلك نحن بحاجة إلى مراجعة اللقاحات المستخدمة في مصر ومحاولة تحضير اللقاحات المحلية مع التقييم الدوري للحماية الشاملة لهذه اللقاحات.