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Molecular identification with sequences of infectious bronchitis virus isolations from broiler chickens in Nineveh

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A B S T R A C T

One of the most dreadful infectious viral disease that cause great economic losses for the poultry industry is the infectious bronchitis IB. The present study conducted to epidemiology and the molecular sequences of infectious bronchitis virus from broiler chickens' fields in Nineveh governorate. The study covered seven regions around the Mosul city included (Al-Msayed, Badoosh, Al-Qayara, Wanna, Basheeka, Al-shalalat , Al-hamdanya, and Bartalla) for the period extends from September 2022 to May 2023**.**

The result recorded 16 suspected cases of IB. 14 of them have been diagnosed as IB virus by means of Polymerase Chain Reaction (Nested PCR). Most of the infections were in October and March, five cases of them were recorded in October, four cases in March, two conditions in September, and one case in other months of the study. (PCR), showed that the causative agent of IB in Al-Msayed, Badoosh, Al-Qayara, Wanna, Basheeka, Al-shalalat , Al-hamdanya, and Bartalla) was from strain(AAS-IB01M Result showed a new strain which was submitted to the GenBank of the (NCBI) to be registered.

Introduction

Infectious bronchitis (IB) is one of the dangerous viral diseases that can affect the poultry industry globally and locally(1), as it causes large economic losses resulting from the rapid spread of the disease, high mortality of the flock and a clear decrease in egg production, (2). Infectious bronchitis virus belongs to the Coronaviridae family and the genus Gamma Coronavirus ,single strand of RNA virus (3).

The common clinical symptoms that can be observed during infection are the rattle of the trachea and respiratory tract, panting, coughing, sneezing, shortness of breath and nasal secretions (4), some cases may affect the reproductive and urinary systems which affecting the quantity and quality of eggs production (5). In previous studies, different strains of infectious bronchitis disease were detected in various regions in the middle, south, and the Kurdistan region of Iraq and these strains which were recorded in flocks of broiler chickens, were Sulaymaniyah Atra (Sul/01/09)(6), and at 2014-2015 the Israeli offspring and QX was recorded in Kufa, Najaf, Dhi Qar, Muthanna, and Basra(7) Then Again, the (Mass) strain was found in Basra at 2015(8), the Polish and South African dynasties found in Najaf and Karbala at 2018 (9). Finally, the strain EU and MK was found in Babylon, Wasit, and Maysan at the years 2018-2019, and the strain which was recorded in Baghdad at the year 2020(10 and 11).

This study was conducted for the purpose of isolation and molecular identification with sequences of IB virus in broiler chicken field in Nineveh governorate.

Materials and Methods:

- **locations and period of the study: -**

The study comprises broiler chickens fields located in Nineveh Governorate in seven rejoins, around the Mosul city which included (Al-Msayed, Badoosh, Al-Qayara, Wanna, Basheeka, Al-shalalat , Al-hamdanya, and Bartalla) for the period extends from September 2022 to May 2023**.**

- **Clinical signs and pathological lesions:**

The clinical signs of the diseased chicks from the suspected cases of the chicken farms which were visited at the rejoins included in the study were recoded then dissection have been done for these chicks to observe the gross pathological lesions of IB infection

- **Polymerase Chain Reaction (PCR).**

PCR technique is done by taking tracheal tissue specimens using the same birds after dissection were frozen in sterile plastic bags for the purpose ofRNA extraction. The process was done using the kit supplied by Add BioInc. South Korea. PCR amplification by using the S1 gene of avian infectious bronchitis virus (IB) using MCE1+ and XCE3- primers utilized by a specific set of primers for each gene as shown in Table (1).

Table 1. The oligonucleotide Primers used for detection of amplification S1 gene of avian infectious bronchitis virus (IB).

Primer	Sequences (5'- 3')	Length	Amplicon size BP	References
MCE1+	AATACTACTTTTACGTTACAC	21	154	
BCE1+	AGTAGTTTTGTGTATAAACCA	21	295	(12)
XCE3-*	CAGATTGCTTACAACCACC	19	---	

* XCE3- is used as reverse primer for both forward primers MCE1+ and BCE1+.

The PCR reaction was made in a 20 µl reaction volume containing 10 µl HS Prime Taq Premix (2X), 5µl cDNA, 1 µl of each reverse and forward primers 10 pmol (Table 1), and the reaction volume was adjusted to the desired volume using 3 µl nuclease-free water. The conditions of PCR assay for *16SrRNA* gene were mentioned in table (2).

Table 2. Steps of the work of the approved PCR reverse transcription program in the detection of infectious bronchitis virus in poultry.

Stage		Steps	Temp(◦C)	Time	Cycles
		Reverse Transcriptase	50	20	1
1st		Initial Denaturation	94	10 min	1
	I	DNA Denaturation	94	45 sec	
2nd	II	Primer annealing *	*53-60	45sec	35
	III	Extension	72	1 min	
3th		Final Extension	72	7 min	1
		Cooling	4	--	--

* The optimum temperature (53°C) at Primer annealing step for SBV-S-382F and SBV-S-469R.
* The optimum temperature (57°C) at Primer annealing step for S1F1 and S1R1.
* The optimum temperature (58°C) at Primer annealing step for S2F2 and S2R.
* The optimum temperature (60°C) at Primer annealing step for SV3F and SV3R.

The electrophoresis assay was used to identify the S1 gene, and 4 µl amplicon was electrophoresis on 1.5% Agarose at 8volt/cm^2 1x TBE buffer for 60 min. DNA ladder sizer TM-100 plus (DNA Invitrogen, USA) was used, then 3 µl of RedSafe Nucleic acid stain was added.

3. Results

3.1 Epidemiology of the disease according to months of the year.

The result registered 16 suspected cases of IB infection distributed in the eight areas of the study. The results of recording cases of infection bronchitis IB in the places included in the study by months show where most of the infections were in October and March, five cases of infection were recorded in October, four cases in March, two conditions in September, and one case each in November, December, January, February, and April. There were no cases of infectious bronchitis reported in the study regions in May as shown in table 3.

Table 3. Prevalence of infection cases according to the months of the study period

May	April	March	February	January	December	November	October	September	The ages of the birds	Infected flocks	Areas	ت
								■	13	18000	Wanna1	1
								■	17	22000	Al-Msayed1	2
							■		27	12000	Basheeka1	3
							■		10	24000	Al-Msayed 2	4
							■		13	16000	Al-shalalat1	5
							■		21	10000	Wanna2	6
							■		9	9000	Badoosh	7
						■			15	12000	Al-hamdanya	8
					■				13	12000	Bartalla	9
				■					17	20000	Al-Qayara1	10
			■						11	5000	Al-shalalat2	11
		■							16	13000	Al-Qayara2	12
		■							9	7000	Al-Qayara3	13
		■							10	4000	Al-shalalat3	14
		■							14	6000	Basheeka2	15
	■								9	11000	Wanna3	16
0	1	4	1	1	1	1	5	2			Total	

3.2 Clinical signs

The Clinical signs observed in this study were mainly in the respiratory system which includes sneezing, coughing, and gasping with lengthening of the neck indicating difficulty in breathing, tracheal rattle, lethargy, wet eyes, foamy secretions from the nose, swollen sinuses, figure(1) lack of feed consumption, with a decrease in the weight of the Chicks, noting that the birds gather under the heat source, and the fluffiness and dirtiness of the feathers were also recorded due to light diarrhea, which is accompanied by a wet mattress. These symptoms vary according to the age of the bird. Also, dead chicks were observed among the flock.

Fig. (1):
A- Signs of sinus swelling and lethargy appear on the infected chick.
B- Signs of difficulty breathing (gasping, open beak) with neck elongation.

3.5 Gross pathological lesions

The majority of consequential gross lesions on chicks suffering from IB infection symptoms were the congestion and mucous secretions in the trachea and observed that there is bleeding in the trachea as shown in Figure (2), with the presence of the caseous plug at the bifurcation of the trachea as shown in Figure (3). There is also congestion and swelling of the kidneys, with accumulation of urea salts in the renal tubules in some cases have been shown in Figure (4).

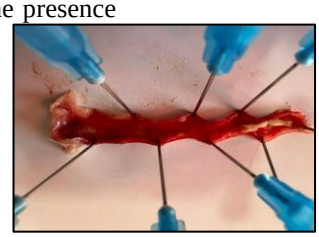

Fig (2). Observation congestion, mucus discharges, and bleeding in the trachea.

Fig (3). Observation of the caseous plug at the bifurcation of the bronchi

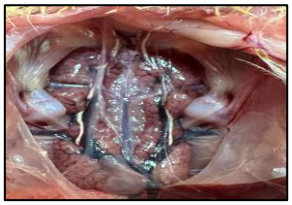

Fig (4). Swelling of the kidneys with the accumulation of urea salts in the renal tubules.

3.3 Results of the diagnosis of infectious bronchitis in poultry by (PCR) technique.

3.3.1 Polymerase Chain Reaction (PCR):

The results of the samples taken from the tissues of the trachea, kidneys, and lung of cases suspected of having infectious bronchitis were shown through the clinical signs and macroscopic lesions of the study areas for the period from September 2022 to May 202) Those were conducted by the polymerase chain reaction (PCR) technique to detect the protein (S1) gene of the infectious bronchitis virus. It was found that (14) fields in the study area showed positive results for the (PCR) examination, while the negative result was on two fields only, which are in the Almasayed, Alshalalat area, as shown in Table (4).

Table 4. Summary of Results of polymerase chain reaction PCR

No.	Areas	No. of chicks in the hall	PCR test
1	Wanna1	18000	+
2	Al-Msayed1	22000	+
3	Basheeka1	12000	+
4	Al-Msayed 2	24000	–
5	Al-shalalat1	16000	–
6	Wanna2	10000	+
7	Badoosh	9000	+

8	Al-hamdanya	12000	+
9	Bartalla	12000	+
10	Al-Qayara1	20000	+
11	Al-shalalat2	5000	+
12	Al-Qayara2	13000	+
13	Al-Qayara3	7000	+
14	Al-shalalat3	4000	+
15	Basheeka2	6000	+
16	Wanna3	11000	+

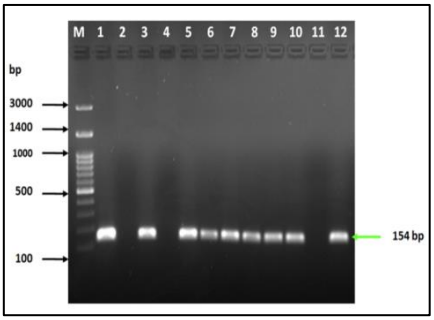

Fig (5): Polymerase chain reaction (PCR) of S1 gene of avian infectious bronchitis virus (IB) using MCE1+ and XCE3- primers. Lane M: 100 bp DNA ladder. Lanes (1, 3, 5 - 10) are positive samples. While (2 and 4) are negative samples, Lane (11) negative control, and Lane (12) positive control vaccine 4/91 strain.

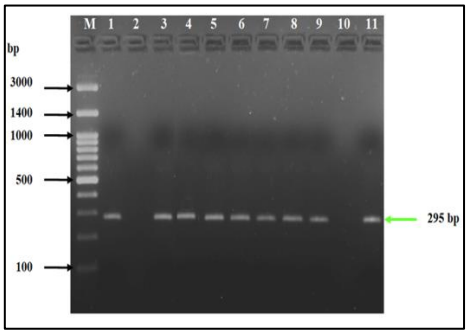

Fig (6): Polymerase chain reaction (PCR) of S1 gene of avian infectious bronchitis virus (IB) using BCE1+ and XCE3- primers. Lane M: 100 bp DNA ladder. Lanes (1, 3 - 9) are positive samples. While (2) are negative samples, Lane (10) negative control, and Lane (11) positive control (vaccine Massachusetts strain).

Table 5. Alignment of multiple gene sequences for regional and global genotypes.

Sample Accession Number	Viral Identified	Query Cover %	Identic Number%	GenBank Accession Number	Country Identification
OQ750697	Infectious bronchitis virus	100	100	KM594197	Morocco
		99	100	MN615459	China
		99	100	MG191031	Thailand
		99	100	MT270489	Saudi Arabia
		99	100	MK887046	China
		99	100	MH427494	China
		99	100	MF447705	China
		99	100	KR265086	China
		99	100	KU145467	Pakistan
		99	100	ON149265	China
		99	100	OK507216	China
		99	100	OM912692	Mexico

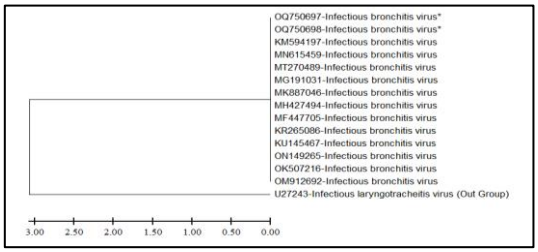

Fig. (7) Phylogenetic tree of gene sequences for regional and global strain.

3.3.2 Results of the molecular diagnosis of infectious bronchitis virus in poultry by DNA sequencing

The result of the molecular diagnosis by genetic sequencing of the amplified fragments of nucleic acid (cDNA) of the infectious bronchitis virus which was collected from the tissues of the trachea, lung, and kidneys, appeared as a positive result in the polymerase chain reaction (PCR). It showed that the causative agent of the study areas in Al-Msayed, Badoosh, Al-Qayara, Wanna, Basheeka, Al-shalalat , Al-hamdanya, and Bartalla) were from strain(AAS-IB01M). It was submitted to the GenBank of the (NCBI) for the purpose of registration as shown in Table (5) and figure (7).

4. Discussion

The recording of cases that were diagnosed in the present study was more common in months October and March compared to the other months. This may be due to the nature of the climate in these months, as it is associated with the fluctuating temperatures causing an increase in stress factors in the intensive breeding fields which induces immunosuppression of the chicks' a predisposing factor for infectious bronchitis(13). Most of the clinical signs that were observed in the current study on chicks suspected of being infected with infectious bronchitis such as (coughing, breathing difficulty, stretching of the neck, rattle, nasal secretions, as well as watery eyes, and swelling of the facial sinuses) are the same symptoms that were observed in other studies such as (14), .These signs and symptoms are corresponded to another study (15). It was observed in all the examined fields that there were general symptoms of the disease such as lethargy and respiratory problems. This was mentioned by the researcher Mahmoud and his group 2019 **(**16**).**

The gross lesions that were observed in infected chicks, for which the anatomical characterization was performed in this study, were marked by the presence of congestion and secretions in the trachea, in addition to the presence of the caseous plug at the bottom of the trachea in the area of the tracheal bifurcation. Furthermore, the presence of congestion in the lungs, enlargement of the kidneys which corresponded with what previously recorded in other studies such as (16 and 17). In the current study, the Nested PCR method was employed as it is more sensitive to detect Coronavirus avian infectious bronchitis virus by analyzing the S1 gene(18). In our study, the results showed that 14 out of 16 fields were positive for PCR, which indicates the spread of the disease in the poultry field in Nineveh Governorate This interpretation showed a high agreement with another study (12).

The reason for the spread of some strains of the infectious bronchitis virus on a large scale from countries of the world, while others remain more local, may be due to the lack of homology in the SI sequence between two strains (such as the vaccinia strain and the field strain), which leads to a greater possibility of large mutations that lead to weakness in cross-protection(19).

The samples were sent to the Korean Macrogen company to confirm the type of strain of the infectious bronchitis virus and the percentage of genetic affinity between the regional and international strains .The results showed that there is a 100% convergence between the approved local strain with the Moroccan, Saudi Arabia, Thailand, Chinese, Mexican, and Pakistani strain.It can be attributed to migratory birds between countries, where the coronavirus was isolated from racing pigeons and many types of wild birds, which may provide a suitable environment for the growth of the virus(20). Additionally, the disease can spread widely to different regions of the world, which creates enormous difficulties in controlling the disease due to the new changing strains(21).

5. Conclusions

The current study established that the incidence of infectious bronchial disease is

correlated nature of the climate different months, as it is associated with fluctuating temperatures, causing an increase in stress factors in the intensive breeding fields, which induces immunosuppression of the chicks'. It is considered a predisposing factor for infectious bronchitis. Registration of a new strain of the virus that causes infectious bronchitis from infections cases diagnosed in Nineveh Governorate which has not being previously registered in Iraq.

References

1-Valastro, V., Holmes, E. C., Britton, P., Fusaro, A., Jackwood, M. W., Cattoli, G., & Monne, I. (2020). S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Since January, 36(January), 1–17.

2- Saif, Y.M., Barnes, H.J., Glisson, J.R., FadlyAM, M. and Swayne, D. . (2003). Diseases of poultry 11thed. Iowa State University Press.

3- King, A. M. Q., Lefkowitz, E. J., Mushegian, A. R., Adams, M. J., Dutilh, B. E., Gorbalenya, A. E., Harrach, B., Harrison, R. L., Junglen, S., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H., Nibert, M. L., Rubino, L., Sabanadzovic, S., Sanfaçon, H., Siddell, S. G., Simmonds, P., … Davison, A. J. (2018). Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). In Archives of Virology (Vol. 163, Issue 9). Springer Vienna. https://doi.org/10.1007/s00705-018-3847-1

4- Lee, E. K., Jeon, W. J., Lee, Y. J., Jeong, O. M., Choi, J. G., Kwon, J. H., & Choi, K. S. (2008). Genetic diversity of avian infectious bronchitis virus isolates in Korea between 2003 and 2006. Avian Diseases, 52(2), 332–337. https://doi.org/10.1637/8117-092707-ResNote.1

5- Cavanagh, D. (2003). Severe acute respiratory syndrome vaccine development: Experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathology, 32(6), 567–582. https://doi.org/10.1080/0307945031000162119 8

6- Mahmood, Z. H., Sleman, R. R., & Uthman, A. U. (2011). Isolation and molecular characterization of Sul/01/09 avian infectious bronchitis virus, indicates the emergence of a new genotype in the Middle East. Veterinary Microbiology, 150(1–2), 21–27. https://doi.org/10.1016/j.vetmic.2010.12.015

7- Seger, W., GhalyanchiLangeroudi, A., Karimi, V., Madadgar, O., Marandi, M. V., & Hashemzadeh, M. (2016). Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. Archives of Virology, 161(5), 1229–1237. https://doi.org/10.1007/s00705-016-2790-2

8- Thwiny, H. T., Mansour, F. T., & Al-Taher, H. A. (2015). Genotypic Characterization of Infectious Bronchitis Virus from Clinically Suspected Broilers in Basrah , South of Iraq. International Journal for Sciences and Technology, 10(4), 54–59. https://doi.org/10.12816/0024586.

9- Nabih Mohammed, R., Bara Allawe, A., Ruya Nabih Mohammed, C., & Nabih Mohammed Atta, R. (2018). Isolation and sequencing of field isolates of Avian infectious bronchitis virus in Iraq. ~ 529 ~ Journal of Entomology and Zoology Studies, 6(1), 529–540.

10- Al-Bawi, F., & Abady, N. R. (2019). Comparative study of phylogenetic analysis of SI gene of infectious bronchitis virus isolates from Iraq with the selected strains (Genebank). Plant Archives, 19, 1904–1908. https://doi.org/10.35248/1948-5964.19.11.183

11- Alhatami, A. O., Alaraji, F., Abdulwahab, H. M., & Khudhair, Y. I. (2020). Sequencing and phylogenetic analysis of infectious bronchitis virus variant strain from an outbreak in egg-layer flocks in Baghdad, Iraq. Veterinary World, 13(7), 1358–1362. https://doi.org/10.14202/vetworld.2020.1358-1362.

12- Isa, R. H., Abdo, J. M., & Al-Barzinji, Y. M. (2022). Genotyping of avian infectious bronchitis virus in broiler farms in Duhok province, north of Iraq. Iraqi Journal of Veterinary Sciences, 36(1), 171–175. https://doi.org/10.33899/ijvs.2021.129635.1670

13- Abdisa, T., & Tagesu, T. (2017). Review on Newcastle Disease of Poultry and its Public Health Importance. Journal of Veterinary Science & Technology, 08(03), 1–7. https://doi.org/10.4172/2157-7579.1000441

14- Emikpe, B. O., Ohore, O. G., Olujonwo, M., & Akpavie, S. O. (2010). Prevalence of antibodies to infectious bronchitis virus (IBV) in chickens in southwestern Nigeria. African Journal of Microbiology Research, 4(1), 92–095. http://www.academicjournals.org/ajmr

15- Seififi, S., Asasi, K., & Mohammadi, A. (2010). Natural co-infection caused by avian inflfl uenza H9 subtype and infectious bronchitis viruses in broiler chicken farms. Veterinarski Arhiv, 80(2), 269–281.

16- Mahmoud,A. M., A. M. S. and A. A. M. E. (2019). The role of infectious bronchitis virus in respiratory and renal problems in broiler chickens. Zagazig Veterinary Journal, 47(1), 32–44. https://doi.org/10.21608/zvjz.2019.6030.1014

17- Yousefi, Y., Bassami, M. R., Kalidari, G. A., & Ghahramani Seno, M. M. (2019). Sequence characterization of full-length S1 gene of infectious bronchitis viruses isolated from poultry farms in Khorasan Razavi, Iran. Turkish Journal of Veterinary and Animal Sciences, 43(2), 235–243

18- Valastro, V., Holmes, E. C., Britton, P., Fusaro, A., Jackwood, M. W., Cattoli, G., & Monne, I. (2016). Infection , Genetics and Evolution S1

gene-based phylogeny of infectious bronchitis virus : An attempt to harmonize virus classi fi cation. MEEGID, 39, 349–364. https://doi.org/10.1016/j.meegid.2016.02.015.

19- Wit, J. J. S. De, Cook, J. K. A., Heijden, H. M. J. F. Van Der, Wit, J. J. S. De, Cook, J. K. A., Heijden, H. M. J. F. Van Der, Wit, J. J. S. De, Cook, J. K. A., & Heijden, H. M. J. F. Van Der. (2011). Infectious bronchitis virus variants : a review of the history , current situation and control measures Infectious bronchitis virus variants : a review of the history , current situation and control measures. 9457. https://doi.org/10.1080/03079457.2011.566260. https://doi.org/10.3906/vet-1808-19

20- Felippe, A. P. A. N., Silva, L. H. A., Santos, M. M. A. B., Spilki, F. R., & Arns, C. W. (2010). Genetic Diversity of Avian Infectious Bronchitis Virus Isolated from Domestic Chicken Flocks and Coronaviruses from Feral Pigeons in Brazil Between 2003 and 2009 Genetic Diversity of Avian Infectious Bronchitis Virus Isolated from Domestic Chicken Flocks. BioOne (Www.Bioone.Org), 54(4), 1191–1196.

21- Hassan, M. S. H., Ojkic, D., Co, C. S., & Cork, S. C. (2019). Delmarva (DMV/1639) Infectious Bronchitis Virus (IBV) Variants Isolated in Eastern Canada Show Evidence of Recombination. Viruses, 5, 1–16.