Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 4139 - 4147
Received 25 April 2021; Accepted 08 May 2021.

Genotyping of Avian Corona Virus from Broilers Infection in Babylon Governorate, Middle Iraq.

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Abstract

This study aimed to track the serotypes of infectious bronchitis virus in poultry Meat farms in Babylon province in the middle of Iraq that are suffering from respiratory symptoms.Fifty poultry flocks were included showing respiratory signs, a rapid examination was performed using the IBV Rapid kit, 13 flocks showed a positive result for the rapid examination, Samples were taken randomly from the kidney and the trachea, from which the RNA was extracted, then converted to complementary DNA cDNA, this last product was entered into a real-time PCR with XCE2+ and XCE2- primers, 7 samples showed a positive result.Special primersMCE1+, BCE1+ and DCE1+ for known pathogenic IBV strains Massachusetts, D274, & 793/B,respectively, were used in a nested PCR, that gave positive results for all three isolates. The flocks were vaccinated with each of the Massachusetts and 793/B strains. Nevertheless, an infection appeared that may be due to the failure of the vaccine, or the immunosuppression. The flocks did not receive the D274 vaccine, and here the issue of introducing them to the vaccination programs must be studied. The isolates that appeared represented the primers, and this does not deny the existence of other isolates, and to prove this, it is recommended to conduct genetic sequencing, which is a future work to complete this study.

Introduction

It is no secret to everyone that the poultry industry is of great importance in supplying the local economy with its products providing job opportunities for several segments of society, in addition to the speed of production compared to other projects (Rossi et al., 2018). This industry has gone through many obstacles that led to its deterioration and caused problems that were a

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reason for the reluctance of many owners of its projects to raise breeding to avoid the economic losses that they may suffer (Kshash & Oda, 2019). Among the most important of these hurdlesare the fatal viral diseases of poultry flocks, infectious bronchitis IB, its importance lies in the fact that it causes a high mortality rate in addition to the losses resulting from the measures taken to avoid the disease represented by periodic sterilizations and boosters as well as the necessary vaccination programs to prevent the risk of infection. Live infectious bronchitis vaccine viruses are widely used by the poultry industry to monitor outbreaks and prevent infections. Vaccine viruses have, however, been implicated in causing outbreaks because viruses isolated from flocks experiencing respiratory outbreaks are often the same serotypes as vaccines (Jackwood & De Wit, 2020), so that, the growth in the poultry sector is being challenged due to increased incidence and re-emergence of diseases caused due to evolution of several viral pathogens and use of live vaccines.

Infectious bronchitis disease caused by avian coronavirus infectious bronchitis virus (IBV), (Walker et al., 2020). IBV envelope contains spike (S) glycoprotein consists of two subunits (S1 and S2) that contains a wide variety of antigenic determinants which may induce the production of specific neutralizing antibodies. Mutations within this genomic region may result in the emergence of new viral variants. IBV's success in the environment is due to its unique capability to evolve. Antigenic variation between strains allows the virus to escape extensive vaccination programs (Gallardo, 2011).Genotyping has been performed using S1 gene sequencing, which has become the procedure of choice for differentiating between vaccine and flock viruses (Seger et al., 2016). .Since the first identification in 1936, more than 50 serotypes or variants of the virus have been reported worldwide. Thus, determining the type as well as isolation of the virus is important to select an appropriate vaccine against IBV infection in the next flock.

Materials and methods

Collectionof the samples

Fifty suspected flocks were visited in Babylon governorate, Samples were collected from flocks that showed clinical and pathological signs related to AIB. The infection was initially confirmed using the IBV rapid kit test. The trachea and kidney tissue specimens were collected and stored directly in tubes containing Triazole and preserved in 2-4°C. The vaccination program for the visited flocks included Mass. and 491 strains.

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RNA extract and conversion to cDNA

RNA was extracted from 13 samples that showed positive results for the rapid kit test. Bioneer/South Korea AccuPrep® Viral RNA Extraction Kit was used, The RNA concentration was measured using the nanodrop, after that the RNA was converted into cDNA depending on the reverse transcriptase by Abm/Canada "OneScript Plus cDNA Synthesis Kit", then the resulting product was preserved at -20°Cuntil it was used.

Detection of IBV by real-time PCR

SYBER green dye (abm/Canada) and specific IBV primers (integrated DNA technology IDT/Canada) were used based onCallison et al procedure (Callison et al., 2006). The test mixture was mixed until 20 µL: Bright Green qPCR Master Mix 10 µl, forward primer 1µl, reverse primer 1µl, cDNA 3µl, and Nuclease-free water up to 20 µl 5µl. The amplification and the detection of a 143-bp fragment of the 5'-untranslated region were done by forward primer 5'-GCTTTTGAGCCTAGCGTT-3', reverse primer 5'-GCCATGTTGTCACTGTCTATTG-3'were used to (5'UTR), and SYBER green dye, the thermal profile was 95˚C for ten min. and (94˚C 15 sec., 50˚C 30 sec., and 72˚C30 sec.) for 40cycles.The device that was used for Real-time PCR is Agilent Technologies/USA.

IBV genotyping by specific nested PCR

A specific nested PCR was employed For genotypic determination of IBV strains by using the specific primers MCE1+, DCE1+, and BCE1+ which specifically amplified a hypervariable region of the S1 gene of Mass., D274, and 793/B serotypes, respectively(Adzhar et al., 1997). Nested PCR was done in two rounds; The final volume of the first round was 20 µL: (13 µL 2X PCR master mix (abm Co.), 1 µL forward primer (XCE2+) primers, 1 µL reverse primer (XCE2-), 2 µL distilled water, and 3 µL cDNA. The thermal profile of the amplification was hot start 94˚C for 5 min., (94˚C for 45 sec., 58˚C for 45 sec., 72˚C for 90 sec. for 40 cycles), and Final extension 72˚C for 5 min. Two microliters of the first-round PCR product were diluted with 198 µL of distilled water. The second round mixtures were performed as in table 2, the thermal profile was hot start 94˚ C for 2min. and 40 cycles (94˚C 15 sec., 48˚C 30 sec., and 68˚C 30 sec.). The sequence of primers is shown in table 2. agarose gel 1% was used for electrophoresis analysis of the final product.

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Table 1 mixtures of the second round of nested PCR

No.	Component			Volume
1.	PCR master mix			13µl
2.	forward primer (XCE3-)			1µl
3.	reverse primer (one of them every assay)			1µl every assay
4.	(DCE1+)	MCE1+	BCE1	
5.	PCR Product from the first round			3µl
6.	D.W.			2µl

Table 2 primers of nested PCR (Adzhar et al., 1997)

primer	Sequence (5`-3`)	Amplification fragment (bp)	
XCE1+ a	CACTGGTAATTTTTCAGATGG	466	1st round
XCE3-b	CAGATTGCTTACAACCACC	466	
XCE3-	TTCCAATTATATCAAACCAGC		2nd round
MCE1+	AATACTACTTTTACGTTACAC	295	
BCE1+	AGTAGTTTTGTGTATAAACCA	154	
DCE1+	TTCCAATTATATCAAACCAGC	217	

Results

Thirteen samples showed a positive result for the rapid IBV kit test. They were selected for the Real Tam test. Seven samples showed a positive result, ie, 53.8%. Positive samples were undergone further analysis to investigate the type of strain, thus nested PCR were performed against three main strains of IBV in which four samples were 1, 2, 4, and 6 to 793/B strain at 150 bp, figure 1 while 1, 2, 3, and 4 in figure 2 were positive for the Massachusetts strain at 295 bp, while the strain D274 showed a positive result in samples 1 and 2 at 217 bp as in figure 3, as appeared in the figures below both sample 5 and 7 gave an unknown result

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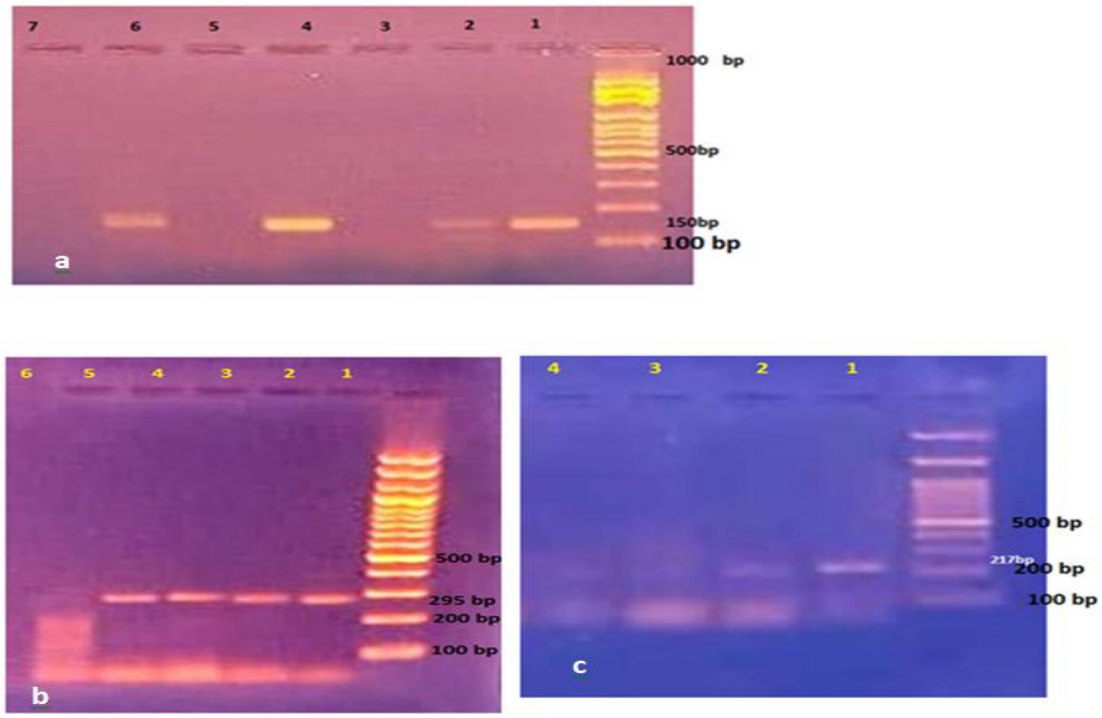

Figure 1 appearance of the results a- at 150 bp in 1, 2, 4, and 6, 793/B strain b- at 295 bp in 1,2,3, and 4, Massachusetts strain c- at 217 bp in 1 and 2, D274 strain

Discussion

AIB disease is important from both an economic and research standpoint, many researchers are obsessed to reach knowledge of the behavior of IBV and its impact on the surrounded conditions adding to the relationship with other diseases, the conundrum of this virus is rapid adaptability against the changing environment.The virulence of IBV vaccines may recur due to its recombination with flock strains (Farsang et al., 2002; Hopkins & Yoder, 1986) this creates new strains that are not subject to the vaccination regime used, as a result of confusing feature which is that cross-immunity between different strains is weak. it is necessary to monitor the changes that may have occurred between the vaccinated and flock isolates (Bochkov et al., 2007).

The results were positive for all the primers of the strains included in the test, as both sample 1 and 2 showed a positive result for Massachusetts, D274 & 793/B, sample 4 positive for both 793/B and Massachusetts, this means that it is possible to be infected with more than one strain at the same time (D Cavanagh et al., 1999; Roussan et al., 2008). It is known that there are two challenges faced by the IBV vaccine, which are the emergence of new strains and the lack of cross-immunity between the different strains (Karimi et al., 2018), however, infection with the

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same vaccination strains Massachusetts and 793/B appeared, here it became possible to add another challenge that may be based on the failure of the vaccine, failure of the vaccination process or inhibition of immune system, which gave an opportunity that the virus used to cause infection.

In the early 1990s, IBVs type 793/B was initially detected in the UK and became one of the prevalent genotypes of Europe in a lot of other countries. (Cavanagh et al., 2005; Dolz et al., 2008), notwithstanding the existence of an attenuated 793/B serotype virus vaccine, recent reports suggest that 793/B serogroup viruses are still widespread in European flock species and the ongoing prevalence thereof remains a major problem in many countries for the poultry sector (Armesto et al., 2011), 793/B was diagnosed in neighboring countries such as Saudi Arabia (Nouri et al., 2003), Iran (Ghalyanchilangeroudi et al., 2019) and Jordan (Roussan et al., 2008), in Iraq, it was found in Sulaymaniyah at 2010 (Mahmood et al., 2011). The initial serotype Massachusetts is now widespread and is used in several marketable vaccines (Benyeda et al., 2010), Mass was the only form of IBV in Israel for many years until 793B was found in 1996 (Jackwood, 2012), in Jordan, mass was identified with Ark and DE-072, and also it appeared with 4/91 and D274 (D A Roussan et al., 2008). D274 isolate of European origin was first diagnosed in the Netherlands in 1984 from Mass-vaccinated flocks (Davelaar et al., 1984), it Appeared in countries near Iraq such as Saudi Arabia (Al-Mubarak & Al-Kubati, 2020), Iran (Ghalyanchilangeroudi et al., 2019) and Jordan (Roussan et al., 2009).

In terms of the presence of strains, the results were partially identical with the results of Seger, Asadpour, and Nouri, except that their studies appeara negative result for the D274 strain (Asadpour et al., 2017; Nouri et al., 2003; Seger et al., 2016), all isolates showed a match with the study (Roussan et al., 2008). D274 strain is not included in the vaccination program for the flocks on which this study was conducted. The failure of the vaccine against Mass. and 491 strains and the absence of D274 in the vaccination program led to the prolonged circulation of these strains in the flocks (Bhuiyan et al., 2021).

The primers are designed to show results for three known strains, there may be other strains that did not appear, to verify more, it became necessary to resort to the genetic sequence to determine the strains in a more correct way. taking into account an important point, which is an attempt to study respiratory diseases as a whole, if not possible, in a greater proportion, i.e. the study is not limited to one disease for the direction of a diagnosis to be accurate, this is if we

accept that the disease may be confined to one organ, but rather to more than one organ, as is known, but a pathognomic lesion maybe that is named and intended for any disease. The emergence of different strains of IBV may be due to the openness that Iraq witnessed to the countries of the world in previous years (Seger et al., 2016), which led to the entry of huge quantities of products and equipment for the poultry industry, both the crowding of the breeding and the high growth ratio may be considered as a stress factor (Davoodi-Omam et al., 2019; Qaid et al., 2016) that is negative on the immune system and thus the outbreak of different diseases.

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