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Avian infectious bronchitis detection in broiler-chickens in Babylon Province

Kifah Rasmi Jasim **Israa Najm Abdullah Al-Ibadi**

Department of pathology and poultry diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Corresponding author: Israa Najm Abdullah Al-Ibadi

Email: Esraa.najm@qu.edu.iq

Abstract

Avian infectious bronchitis (AIB) causes economic losses in many flocks of poultry; the causative agent is the avian coronavirus which belongs to coronaviridae that causes multiple diseases in the human and animals. AIB is one of the main causes of economic harm; it causes respiratory disease, lesions, as well as decline in egg production with deformity. Infectious bronchitis virus IBV has many serotypes without creating cross-protection against each other. This study aimed to detect IBV infection in broiler flocks in Babylon province depending on the clinical symptoms, postmortem lesions, IBV Rapid kit, and real-time polymerase chain reaction rt-PCR. Fifty broiler flocks were shown respiratory signs, a rapid examination was performed using the IBV Rapid kit, 13 flocks revealed positive results, Samples were collected from the trachea and the kidney for RNA extract then complementary DNA cDNA synthesis, the result-product entered a real-time PCR with XCE2+ and XCE2- Primers, only 7 samples showed positive results.

Keywords: Broiler, Coronavirus, Infectious bronchitis.

Introduction

Coronaviruses are widely spread in the world, highly contagious, and incredibly infectious. These can cause respiratory, enteric, and hepatic disease in some cases and neurological disorders in a wide variety of animals and humans (1). Infectious bronchitis virus, the prototypic coronavirus, has a

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significant economic effect on the poultry industry, resulting in a loss of millions of dollars per year worldwide due to the rejection of chickens contaminated with the virus (2). It is difficult to control on disease because they have a broad genetic diversity, weak cross-protection between the different strains, and a high mutation rate (3). Vaccination programs are widely used by the poultry industry to monitor outbreaks and prevent infections (4). Vaccine have, however, been implicated in causing outbreaks because viruses isolated from flocks experiencing respiratory outbreaks are often the same serotypes as vaccines (5). Antigenic variation between strains allows the virus to escape extensive vaccination programs (6). There are several diagnostic methods to distinguish IBV some of them give a suspected result, such as clinical signs and post-mortem, to confirm this, laboratory methods such as detection by PCR or genetic sequencing are relied on.

Materials and methods

Investigated flocks

Fifty flocks were visited in Babylon Governorate during the period from October 2020 to February 2021, chickens of flocks suffered from respiratory signs with high mortality. All birds were vaccinated with IBV vaccine H120, 491 vaccine strains.

Primary diagnosis

Both clinical signs and post mortem finding were relied upon the first expected diagnosis of infectious bronchitis (IB) in the visited flocks.

Detection of IBV by rapid kit

A deleted rapid kit was used to confirm the primary diagnosis of the infected flocks; swabs were taken from the trachea and placed in a buffer solution. It was mixed well then the mixture was placed in the special hole in the cleft, the result was reported after 15 mins. Samples were taken from the kidney and the trachea was placed in a tube containing 1 ml of triazole and kept at 4 ° C refrigerated until use.

RNA extract and conversion to cDNA

Using a special kit from a company Bioneer/South Korea AccuPrep, the RNA was extracted from the samples and then converted to cDNA using the “OneScript Plus cDNA Synthesis Kit Abm/Canada. The product was then preserved in the freeze at 20°C.

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Detection of IBV by real-time PCR

The real-time test was performed on the samples using the SYBER dye technology (7), SYBER green dye (abm/Canada), and specific IBV primers (integrated DNA technology IDT/Canada), the mixture was Bright Green qPCR Master Mix 10 µl, forward primer 5'-GCTTTTGAGCCTAGCGTT-3' 1µl, reverse primer 5'-GCCATGTTGTCACTGTCTATTG-3 1µl, cDNA 3µl, and Nuclease-free water up to 20 µl 5µl So that it became the sum of the mix 20 µL. The test was performed with the following temperature setting profile 95˚C for 10 min. and (94˚C 15 sec., 50˚C 30 sec., and 72˚C 30 sec.) for 40 cycles, Agilent Technologies/USA device was used to conduct the work

Results

Respiratory symptoms such as sneezing, coughing, dyspnea, and ralse, as well as lachrymation and nasal secretions, in addition to Emaciation, lack of activity and congregation of birds around the source of heat, poor feed intake and water drinking, and a clear decrease in weight were observed in the examined flocks.

The trachea is the most affected part represented by inflammatory secretions with obvious congestion that may reach the stage of hemorrhage, figure 2. At this point of infection, the name or tropism of the virus is a respiratory infection, but it may infect other organs, the most important of which is the urinary and reproductive system. The plug appeared in the bifurcation of the trachea figure 3, as a result of secretions collecting in it with fibrin accumulation around both the heart (pericarditis) and the liver (perihepatitis) as the result of secondary infections. The kidneys were enlarged and congested with the accumulation of urea salts in the renal tubules figure 4

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Figure (1): congested and hemorrhagic trachea

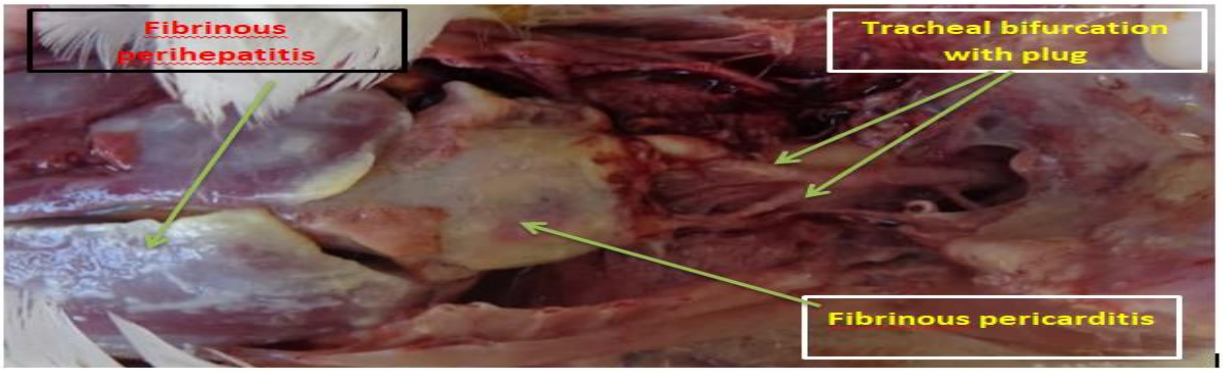

Figure (2): tracheal plug plus, fibrinous perihepatitis, and pericarditis due to complicated bacterial infection as *E. Coli* and/or mycoplasma infection

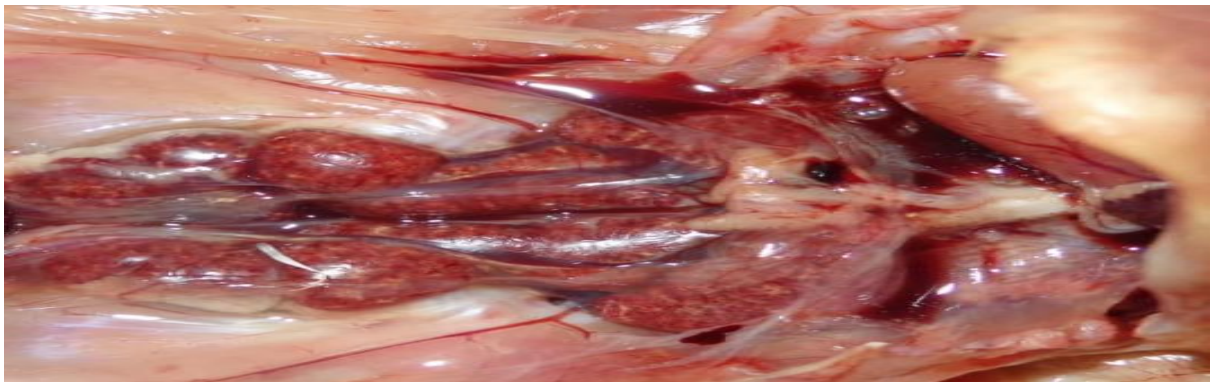

Figure (3): urate deposition in renal tubules with enlarged and congested kidney

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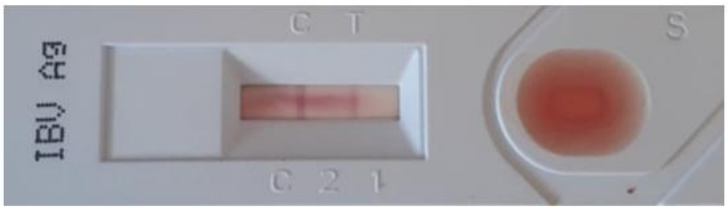
The results showed 13 positive samples for the rapid kit test figure 5, which were then, used into the molecular test by extracting RNA and making cDNA. In the rt-PCR, seven samples showed a positive result as shown in figure 6.

a-



b-

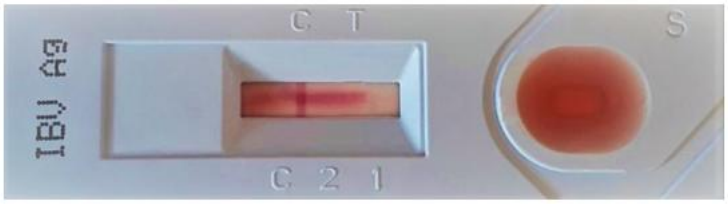


Figure 4 rapid kit result of IBV in the positive result (a) the indicative line appeared at C and T portions, while in the negative result (b) the indicative line appeared at C portion only

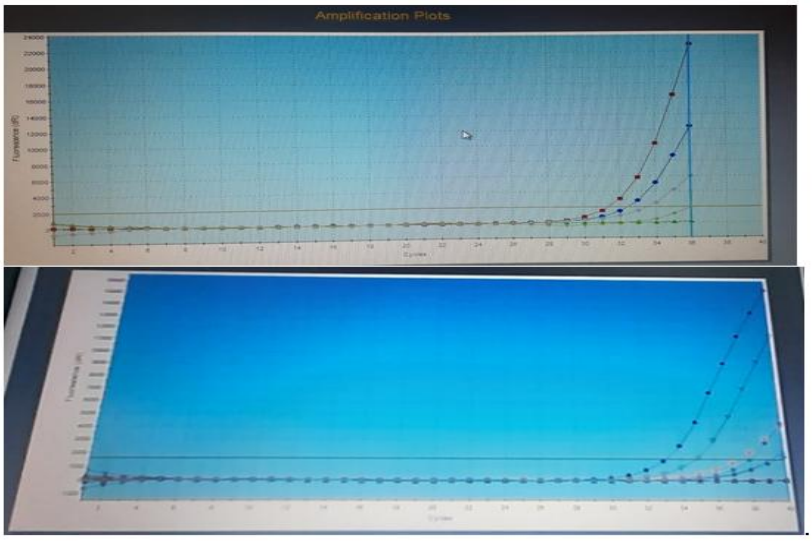


Figure 5 results were seven samples showed a positive result between all samples

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Discussion

AIB disease is significant in economics as well as in science; many investigators are concerned with acquiring knowledge of IBV's actions and its effect on the surrounding environments contributing to relationships with other diseases . IBV vaccinations can recur because of their recombination with flock strains (8,9). This introduces new strains not under the vaccine system used; As a consequence of the confusing characteristic that cross-immunity is low. The changes between vaccinated and flock isolates that might have happened must be monitored (10). A number of researchers have discussed the disease in Iraq, where it has been proven that there are a number of strains; this is summarized in Table 1 below.

Table 1 IBV strains in iraq

Strain	Years	Referance
IS/1494 (EU350551.1)	2014–2015 2020	1- (11) 2- (12)
793/B (DQ294723.1)	2014–2015	
QX (MN548289.1)	2014–2015	
DY12-2 (KU143901.1)	2014–2015	
MK562377	2018	(13)
MK562376	2018	
MK562375	2019	
MK562374	2019	
CK/CH/LDL/97I (JX195178.1)	2012	(14)
Poland KY047602.1	2017	(15)
Western Africa FN430414.1		
Mass	2015	(16)
GQ169242 (IBV/Brazil/PR05)	2017	(17)

The starting point of the disease is the respiratory signs (5). As shown by the clinical signs and post mortem, the most important and clear sign of the disease is congestion of the trachea, which developed and extended to be an inflammatory exudation, which was the cause of tracheal obstruction, especially in the tracheal bifurcation (18) which lead to the death of the bird due to the asphyxiation. Another cause and lesion that may threaten the

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flocks with death is the kidney infection that causes kidney failure and the deposition of urate salts (19) . The use of IBV rapid Kit showed results when compared with the results of rt-PCR, the ratio was 53.8% that may be low and unlikely for accurate flock diagnosis. Using of rt-PCR is characterized by a quick reading of the results and knowing the progress of the reaction by monitoring the test path on the computer screen, adding to it can sense nucleic acid with the lowest concentration when compared to the traditional method, it is faster and does not require electrophoresis (20), this is if the financial aspect is not considered, as rt-PCR is more expensive. The disease is present and diagnosed in many countries, including Iraq and neighboring countries, with different strains and always developed (21) it needs periodic monitoring, relying on all available flock and laboratory methods. Reliance on disease control is heavily dependent on preventive immunization (4) taking into account the genetic diversity obtained. Here is a point that should not be overlooked, which is the management side with all its details.

It was noticed when visiting the flocks many gaps and lapses that need to be corrected, the most important point in which is ventilation, heat, and their problems, in addition to mycotoxins and their relationship to immunosuppression, which effectively leads to the failure of the vaccine and thus the loss of vaccine expenses with the infection of the flock. The results of the study to this extent are diagnostic for the presence of the virus, and here as a future work that needs to be supplemented with the investigation by detecting virus strains using the genetic sequence to determine the pathological strains and their linkage with the vaccine strains by developing vaccination programs including those strains to simulate the pathogenic strains.

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