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Diagnosis of Infectious Bronchitis Infection in Broiler Chicken Farms in Salah Al-Din Governorate

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Abstract

THE study aimed to diagnose the infection of infectious bronchitis in broiler chicken farms in Salah Al-Din governorate. Using the Infectious Bronchitis Virus **(**IBV) Rapid antigenic test and Polymerase Chain Reaction, The study included eight neighbouring regions for the city of Tikrit: (Al-Dabbssa, Al-Naaeimah, Al-Khazifei, Al-Alam, Sammrah, Al-Khzammeah Al-kharja and Al-Hammrah) during the period from October 2022 to February 2023. The results of our study showed diagnosis cases of infection with the disease in all regions and the infection rate was higher in regions Al- Naaeimah, Al-Khazifei and Al-Khzammeah. In addition, the infection rate was higher in October compared to other months. The results also showed the Infectious Bronchitis Virus (IBV) Rapid antigenic test gave real diagnostic results for infection with the disease. The study concluded that infection with the disease is present in all regions included in the study, and the infection rate was higher in three regions compared to the other regions. It was also shown that the weather has a role in the spread of infection during a certain time. In addition, the results showed that the IBV Rapid antigenic test can be relied upon in diagnosing cases of infection. disease because it gives real diagnostic results.

Keywords: Infectious bronchitis, diagnosis broiler chickens, Salah Al-Din governorate.

Introduction

One of the most dangerous viruses that can infect chickens is the avian infectious bronchitis virus, or IBV. In light of this virus, the chicken industry faces difficulties in many regions of the world [1]. The prototypical coronavirus known as the infectious bronchitis virus causes millions of dollars' worth of annual losses for the poultry industry globally [2,3]. Infectious bronchitis affects birds of various ages and results in respiratory problems such as breathing difficulty, tracheal rattling, panting, coughing, and sneezing with or without facial swelling, frothy discharge in the eyes, nasal mucous discharge, and general weakness, with the young chicks huddling under the heat sources [4,5]. Some IB variant strains affecting the urinary system, especially the kidneys [6,7]. Various virus serotypes from broiler infections have been identified in various parts of the middle and south of Iraq as well as the Kurdistan province. The disease was first noted in Sulaimani in 2011 [8]. In 2018 the disease was recorded in Baghdad and Babylon, in 2019 IB cases in Wasit and Mesan provinces, in 2020 IB infection was confirmed in Baghdad [9] Various tests, such as virus neutralization, hemagglutination inhibition, ELISA, RT-PCR, and virus isolation, have been used to track the various IBV serotypes that are currently present [10,11]. There are different diagnostic techniques for identifying IBV. But some tests may be providing a suspected result, such as post-mortem and clinical signs, therefore PCR detection or genetic sequencing, are used to confirm diagnosis.

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Material and Methods

Broiler farms included in the study

Broiler farms in eight regions surrounding Tikrit City were included in the study:

Al-Dabbssa, Al- Naaeimah, Al-Khazifei, Al-Alam, Sammrah ,Al-Khzammeah Al-kharja and Al-Hammrah .

Diagnostic examination

The study involved visiting the broiler farms and obtaining tracheal and kidney samples from 24 birds (3 birds from each region) and collecting tracheal swabs (68 swabs for five months) from different regions that were exhibiting clinical signs of infectious bronchitis with present the mortality during the period between October 2022 to February 2023. Owners of flocks was administered to all birds by IBV vaccine strains (H120 and 4/91).

Laboratory diagnostic techniques consist of:

IBV detection by using a rapid antigenic kit test

Collected the swab samples from trachea were put in the buffer solution to validate the initial diagnosis of the infected farms for using detected rapid antigenic kit test. After that, the mixture was mixed well and placed in the designated slot in the designated place, and then the results appeared after 15 minutes. The samples of Kidneys were extracted, and the trachea was added in the tube with one milliliter of triazole. The tube was then refrigerated at 4°C until it was needed.

Extracting RNA and turning it into cDNA

The RNA was extracted from the samples using a specialized kit produced by the company of Bioneer / South Korea Accu Prep, and the One Script Plus for cDNA Synthesis Kit Abm / Canada was used to convert RNA to cDNA. After that, the product was kept frozen at -20°C.

IBV detection by using real-time PCR

Birds collected from the farms that visited to obtain samples (trachea and kidneys) were dissected and then frozen in sterile plastic bags for RNA extraction using the kit provided by Add Bio Inc. South Korea. One -step reverse transcription of RNA to cDNA was performed and PCR cycling conditions for amplification of infectious bronchitis (IB) virus were described as described below:

Using SYBER dye technology [12], the samples were put through a real-time test. Bright Green qPCR Master Mix was the combination made up of specific IBV primers (integrated DNA technology, IDT / Canada) and SYBER green dye (abm / Canada). 10 microliters, 1 microliter of the forward primer 5'-CGTTTTCACGCTACGCTT-3', 1 microliter of the reverse primer 5'-CGCATCTTGTGACTGTCTATTG-3, 3 microliters of cDNA, and 20 microliters of nuclease-free water for it to equal the mix's 20 µL. Using an Agilent Technologies / USA device, test was conducted using the profile of temperature settings listed below: 95 ˚C for ten minutes. with (94 ˚C fifteen seconds, 50 ˚C thirty seconds, and 72 ˚C thirty seconds) for forty cycles.

Results and Discussion

The clinical signs observed included loss weight, depression, loss of appetite, ruffled of feathers, the closing of the eyes, lameness, difficulty breathing with the beak open, dehydration, fainting, and death, and these signs are consistent with what the researchers 13].

The study recorded Congested and hemorrhage in the trachea (Figure 1). This result concurs with [14]. Also, present is congestion and enlargement the liver with fibrinous pericarditis (Figure-2) this concurred with [15,16]. Pericarditis and air saculitis (Figure 3), that concur with [17]. Kidney enlargement and congestion with urate retention in the ureters (Figure-4) concur with [16]. The cause of kidney enlargement may be a result of the accumulation of uric acid crystals in the renal tubules, which leads to an increase in the size of the kidney. Also, the cause of the accumulation of uric acid crystals in the ureters results from the failure of the urinary system to get rid of uric acid, and thus the crystals accumulate in the ureters.

While the study Elucidate infiltration the inflammatory cells in the trachea (Figure 5) this concur with [15]. Also, the study Elucidate Inflammatory cell infiltration with enlargement and degenerated of glomerulus in the kidney for infected broiler (Figure- 6) this result concur with [18].

IBV detection by using a rapid kit:

The rapid test detection results for the chicks with respiratory disorders, nasal discharge, and increased farm mortality rate, which was exhibiting clinical signs of IB, indicate that all cases tested positive for the trachea samples (Figure- 7), negative for 15 out of 24 kidney samples, and positive for three each from Al- Al- Naaeimah, Al-Khzammeah and Al-Khazifei (Table 1).

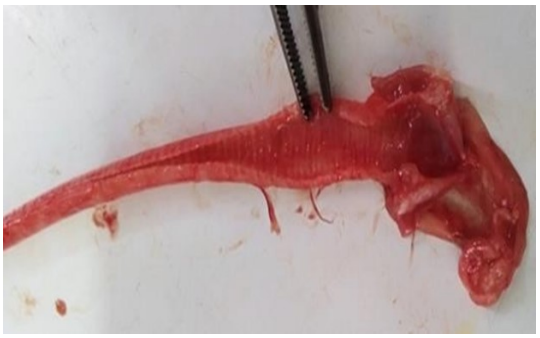

Fig. 1. Congested and hemorrhage in the trachea

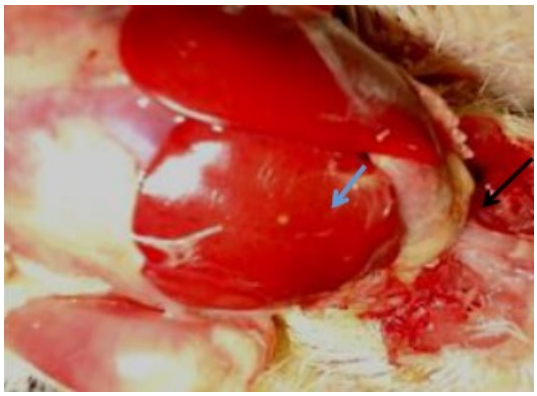

Fig. 2. Congestion and enlargement of the liver (Blue arrow) with fibrinous pericarditis (Black arrow)

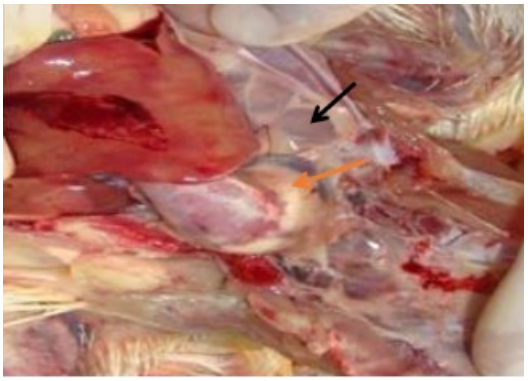

Fig. 3. Showed Pericarditis (Orange arrow) and airsaculitis (Black arrow)

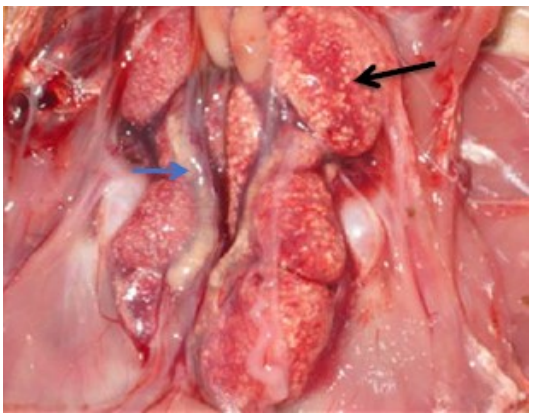

Fig. 4. Kidney enlargement and congestion (Black arrow) with urate retention in the ureters (Blue arrow).

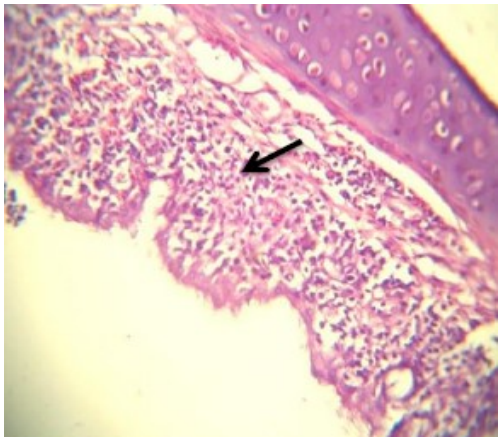

Fig. 5. The histological section of the broiler chickens' trachea reveals infiltration of inflammatory cells (H&E) 100X.

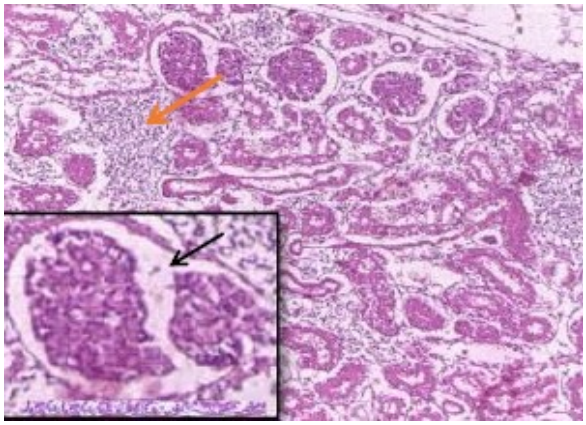

Fig. 6. Inflammatory cell infiltration (Orange arrow), enlarged degenerated glomerulus (Black arrow). (H&E) 40x.

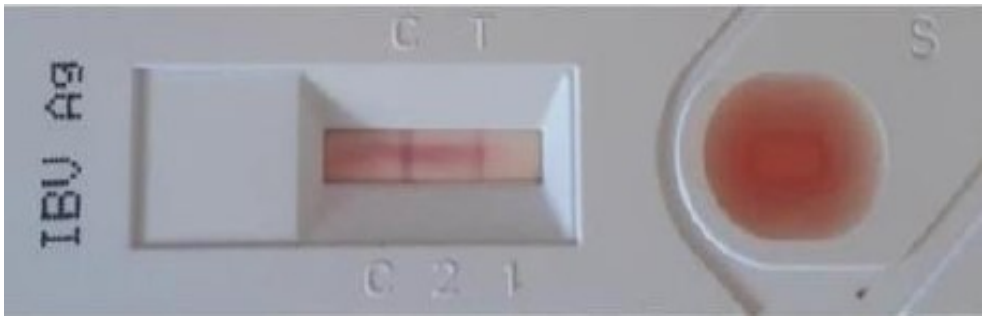

Fig. 7. Showed the positive IBV rapid kit result.

TABLE 1. Displays the Antigen Rapid Test results by age and region.

No. of cases	Regions	The chicks' age (in days)	No. of infected farms	Rapid test (kidney)	Rapid test (trachea)
1,2,3	Al-Dabbssa	15	1	-	+
4,5,6	Al- Naaeimah	14	1	+	+
7,8,9	Al-Khazifei	17	1	+	+
10,11,12	Al-Alam	20	1	-	+
13,14,15	Sammrah	14	1	-	+
16,17,18	Al-Khzammeah	16	1	+	+
19,20,21	Al-kharja	19	1	-	+
22,23,24	Al-Hammrah	21	1	-	+

Rapid test kit results for suspected cases of infectious bronchitis were compared across the study regions during the five months. It was observed that Al-Al- Naaeimah, Al-Khzammeah and Al-Khazifei had higher IB incidences and that October had more infection rate overall showed table .2.

TABLE 2. Describes prevalence of Infectious Bronchitis Virus infection according to the month.

The total number for tracheal swabs	October	November	December	January	February
	Positive samples	Positive samples	Positive samples	Positive samples	Positive samples
68	12 (%17.64)	9 (% 13.23)	7 (% 10.29)	5 (% 7.35)	7 (% 10.29)

We observe cases of IB infection even after most broiler farms implemented a scheduled IB vaccination program. Twenty four sample cases with particular clinical indicators of IB infection were noted in this study. Reason of higher number of Al-Naaeimah, Al-Khzammeah and Al-Khazifei had a higher number of suspected IB cases than the other study regions; this could be because these regions have a higher concentration of broiler farms.

October has a higher rate of IB infection than the other months. This could be because of the high temperature fluctuations in the environment, which reduce broiler chick immunity and act as a predisposing factor for IB infection even in the presence of proper IB vaccination [19,20,21]. The tracheal samples positive results and the kidney samples negative results suggest that the disease was most likely in its early stages. The Antigen Rapid Test results for two of the samples were negative, indicating that there is despite clinical indications of infection, there was no IBV infection. Newcastle disease and other non-IB infections could be the cause of this. [22]. additionally, the PCR is the most effective confirmatory test for the diagnosis [23]. The PCR results for fifteen of the twenty four samples were positive, indicating that the disease is spreading throughout the Salah Al-Din governorate's poultry farms. Several (PCR) techniques have been developed to analyze the S1 gene and determine the prevalence of the infectious bronchitis virus [24].

IBV detection by using real-time PCR

The tissue samples' PCR results show that eight of the flocks were infected with the IB since they produced positive results for both the vaccine Massachusetts strain (Fig. 8) and the lane positive control vaccine 4/91 strain (Fig. 9).

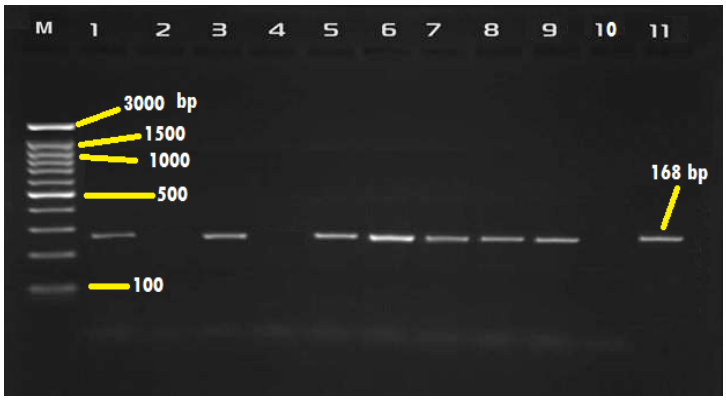

Fig. 8. MCE1+ and XCE3-primers are used in the polymerase chain reaction (PCR) of the S1 gene of the avian infectious bronchitis virus (IB). 100 bp DNA ladder in Lane M. Samples in lanes 1, 3, 5, and 9 are positive. Lane 10 is the negative control, Lane 11 is the positive control (vaccine 4/91 strain), and samples 2 and 4 are negative.

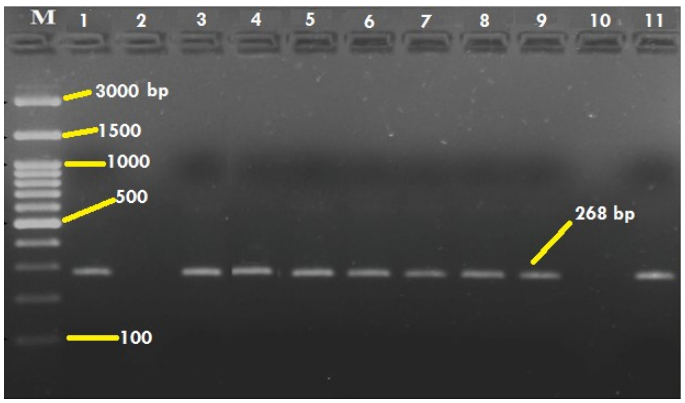

Fig. 9. BCE1+ and XCE3-primers are used in the polymerase chain reaction (PCR) of the S1 gene of the avian infectious bronchitis virus (IB). 100 bp DNA ladder in Lane M. Positive samples are found in lanes 1, 3–9. Lane 11 is the positive control (vaccine Massachusetts strain), Lane 2 is the negative sample, and Lane 10 is the negative control.

RT-

PCR is sensitive and frequently used to differentiate the amplicons obtainedit was used in this study [25,26]. The study appeared that samples tested positive result for the vaccine strains could indicate that the flocks had already contracted IBV before being vaccinated, that the vaccine was mixed with different strains, or that there had been recombination between the field and vaccine strains [27,28]. The samples' positive responses to both vaccine strains are consistent with the findings of [27,28]. study, which discovered that both the (Massachusetts and 4/91) strains were present in 24 tested farms. This finding may indicate that there was little to no cross-protection between the two vaccines against the infectious bronchitis field virus.

Conclusions

The study concluded that infection with the disease is present in all regions included in the study, and the infection rate was higher in three regions compared to the other regions. It was also shown that the weather has a role in the spread of infection during a certain time. In addition, the results showed that the IBV Rapid antigenic test can be relied upon in diagnosing cases of infection. disease because it gives real diagnostic results.

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Conflict of interest

There are no conflicts of interest to be declared.

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Author contributions

Conceptualization, study design, sample collection, data analyses, Manuscript drafting, and manuscript finalization: Abduljabbar M. H. Aljubori and Qusai S. Jumma.

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تشخيص الإصابة بالتهاب الشعب الهوائية المعدية في مزارع الدجاج اللاحم في محافظة صلاح الدين

عبدالجبار محمد حسين الجبوري وقصي صالح جمعه

فرع الامراض وامراض الدواجن - كلية الطب البيطري - جامعة تكريت - العراق.

هدفت الدراسة إلى تشخيص الإصابة بالتهاب الشعب الهوائية المعدية في مزارع الدجاج اللاحم في محافظة صلاح الدين. تم استخدام اختبار المستضد السريع IBV وتفاعل البوليميراز المتسلسل، وشملت الدراسة ثماني مناطق مجاورة لمدينة تكريت وهي: (الدبسة، الناعمة، الخزيفي، العلم، سمرة، الخزامية، الخرجة، والحمرة) خلال الفترة من تشرين الأول 2022 إلى شباط 2023. حيث أظهرت نتائج دراستنا تشخيص حالات إصابة بالمرض في جميع المناطق، وكانت نسبة الإصابة أعلى في مناطق الناعمة والخزيفي والخزامية. بالإضافة إلى ذلك، كان معدل الإصابة أعلى في شهر أكتوبر مقارنة بالأشهر الأخرى. كما أظهرت النتائج أن اختبار المستضد IBV Rapid أعطى نتائج تشخيصية حقيقية للإصابة بالمرض. وخلصت الدراسة إلى أن الإصابة بالمرض موجودة في جميع المناطق التي شملتها الدراسة، وكانت نسبة الإصابة أعلى في ثلاث مناطق مقارنة بالمناطق الأخرى. كما تبين أن الطقس له دور في انتشار العدوى خلال فترة زمنية معينة. بالإضافة إلى ذلك، أظهرت النتائج أنه يمكن الاعتماد على اختبار المستضد IBV Rapid في تشخيص حالات الإصابة. المرض لأنه يعطي نتائج تشخيصية حقيقية

الكلمات المفتاحية: تشخيص الاصابة بالتهاب الشعب الهوائية ، الدجاج اللاحم، محافظة صلاح الدين.