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Research Article
Serological Survey and Molecular Detection of Infectious Bronchitis Virus in Broiler Chickens in Diyala Province, Iraq

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Abstract

Background and Objective: Infectious Bronchitis (IB) is considered one of the highly important contagious viral diseases of poultry worldwide. This study is carried out to determine the prevalence of IB in commercial broiler chickens in Diyala province, Iraq. **Materials and Methods:** The study was carried out during the period of August, 2014 until December, 2014. Swabs samples (n = 200) were collected from 20 flocks with a history of birds showing clinical signs suggestive of Infections Bronchitis Virus (IBV) infection. Samples were analyzed by immunochromatographic test and Reverse Transcription-polymerase Chain Reaction (RT-PCR). **Results:** About 77.5% (155/200) of the swabs samples were found seropositive for IBV infection. Whereas RT-PCR analysis using primers specific for the detection of IBV M gene showed that 67.5% (50/74) of the samples collected from 20 flocks were positive for IBV. **Conclusion:** It was concluded that the prevalence of IB is high among chickens commercially raised in the Diyala province of Iraq. Future molecular studies are needed to understand the genotype as well as the genetic diversity of IBV viruses found in the province to guide the choice of vaccines for prevention and control.

Key words: Chicken, infectious bronchitis virus, immunochromatography, RT-PCR, Diyala

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Avian Infectious Bronchitis (IB) is an acute and highly contagious disease of poultry caused by Infectious Bronchitis Virus (IBV). The causative virus is an RNA virus which belongs to the family coronaviridae under the order Nidovirale[1]. The disease affect the respiratory tract, kidney and reproductive organs, thus causing huge economic loss as a result of conditions such as decrease in egg production, poor carcass weight, morbidity and mortality[2]. The first incidence of the disease was reported in 1930s in North Dakota in United States of America. However, IB continue to be an important devastation in poultry worldwide[3-5]. Although generally considered as a problem in commercial chicken, many birds within the galliform family have been reported positive for the disease. The disease usually might lead to high morbidity in all ages of chickens, however, this is more pronounced in chicks less than 3 weeks of age, thus young chicks are more at risk for the disease and therefore need immunization either at hatchery or before 3 weeks of age[6,7].

Three manifestations of IBV infection are generally observed in the field, namely the respiratory, gastrointestinal and urogenital forms[8]. The respiratory form is characterized by respiratory distress manifested in form of coughing, tracheal rates, gasping and sneezing and excessive mucus production in the bronchi. On the other hands, nephropathogenic form of IB clinically cause depression, wet droppings and increased water intake while enteric form is often restricted merely by diarrhea[8,9]. Initially, it was believed that all the isolates belong to a single prototype termed Massachusetts (Mass) serotype mostly isolated from commercial poultry[6]. Subsequently, it was found that the virus has the tendency to mutate frequently due to lack of error-proof mechanism that often lead to a huge variability in the S1-glycoprotein gene. Today more than 60 IBV genotypes are said to emerge and most of the newly identified genotype are not cross protected by the commonly available commercial vaccines such as the American Mass and European 4/91 serotypes derived vaccines. In addition, new IBV variants may produce severe effect in terms of pathogenic outcome thus leading to an increased morbidity and mortality rates[8]. The rate at which new virus strains emerged couple with the lack of cross-protection therefore warrant continues surveillance for the emerging viruses so as to understand the molecular epidemiology of IB and to possibly produce new generations vaccines that are effective for the control and prevention of the disease[10-12]. In Iraq, IB is still one of the most serious challenges yet little information is known on molecular evidence of IB in the province of Diyala, which is located in the eastern governorate of Iraq. The purpose of this study therefore was to investigate the serological and molecular evidences of IB in selected broiler chicken's flocks in Diyala governorate, Iraq.

MATERIALS AND METHODS

Samples collection: Samples were collected from 20 infected broiler flocks showing clinical signs including depression, severe respiratory signs, mild to serious nasal discharges and occasional but infrequent gasping. Grossly there was severe congestion and cast plug in the bifurcation of the trachea, pale and enlarged kidney.

Immunochromatography assays: Swab samples taken from cloaca of the affected chickens (feces) were collected from 20 chicken flocks (10 chickens per flocks) and screened using commercially available Rapid IBV Antigen Test Kit ((BIONOTE, Incorporation, Seoul, South Korea) according to the manufacturers guide. Briefly, 3 drops of supernatant from the homogenized and centrifuged fecal suspension were placed into the sample hole on the test device. The test was observed for the appearance of purple color moving across the result window at the center of the test device and the test results was considered positive if there was colour change in the positive-test-indicator line. All steps were completed and read within 10 min period.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR): For molecular detection, tissue samples from lungs and trachea were collected in sterile plastic test tubes and stored in deep freezer at (-20°C) in the Laboratory of Microbiology, College of Veterinary Medicine, University of Diyala, until used. Viral RNA was extracted from lungs and trachea tissues using Trizol Reagent (Invitrogen, USA) according to the manufacturer's instructions with minor modification. Briefly, 2000 µL of Trizol reagent was added to 200 g of tissue (lungs or trachea) and incubated at 20°C for 10 min. About 400 µL of chloroform was then added and vigorously shaken for 20 sec prior to incubation at 20°C for 5 min. The mixture was then centrifuged at 12,000×g for 15 min at 4°C (MIKRO 22R, Hettich, Germany). Following the centrifugation, the mixture was observed to separate into a lower red phenol chloroform phase, interphase and the colorless upper aqueous phase. The aqueous phase was carefully transferred to a fresh 1.5 mL microcentrifuge tube and RNA was precipitated by mixing this part with 600 µL of cold isopropanol and left for 10 min at room temperature. Further centrifugation was carried out at 12000 g for 10 min and the supernatant discarded before

washing the RNA pellet with 1 mL 0f 75% ethanol. During washing, another centrifugation was carried out at 7500 g for 5 min at 4°C. Ethanol-washing step was repeated with 1 mL of 100% ethanol, the supernatant was again discarded and the resulting pellet was briefly dried prior to resuspension in 20 µL of RNAse-free water (Promega, USA). Samples were aliquoted and stored at -70°C until use.

The following RT-PCR condition was used to amplify IBV M-gene segment in a single-tube assay with a final reaction volume of 25 µL using commercially available kit (iNtrON Biotechnology, Korea). The PCR reaction consists of 20 µL of RT-PCR premix (OptiScript™ RT system, RT-PCR buffer, i-Star *Taq* TM DNA polymerase, dNTPs, chemical stabilizer, 8-MOP (dissolved in DMSO)), IBV-M-gene specific primers. A total of 2 µL of template RNA was added into the RT-PCR premix tube and followed by addition of 5 µL of nuclease free water with gentle mixed mixing. A positive control tube was used for comparison with test samples. The RT-PCR tubes were quickly spun for a few seconds and transferred to a thermal cycler (Eppendorf, USA) set for 42 cycles as shown in Table 1.

Following amplification, PCR product (about 7 µL) was analyzed by electrophoresis in a 1.5% agarose gel stained with RedSafe acid solution. Electrophoresis was set and carried out at 100 V for 30-40 min and DNA product visualized using ultraviolet (UV) trans-illuminator in the presence of 100 bp DNA ladder.

Table 1: Amplification program used of the real-time PCR assay

RT-PCR		Temperature (°C)	Time
1Cycle	Reverse transcription reaction	45	30 min
	Inactivation of reverse transcriptase	94	5 min
40 Cycles	Denaturation	94	30 sec
	Annealing	52	30 sec
	Extension	72	40 sec
1Cycle	Final extension	72	5 min

Table 2: Infection bronchitis virus seropositive rates obtained from farms in different regions of Diyala, Iraq

		Positive samples of bird/flock	
Location	No. of birds check	No.	%
Baqubah	50	40	80
Al-Mokdadia	20	16	80
Kanan	40	28	70
Baladrose	40	32	80
Al-Kales	30	21	70
Al-Mansoria	20	18	90
Total	200	155	77.5

Overall seropositive rate = 77.5%

Table 3: Result of infectious bronchitis (by RT-PCR) according to the area

	Birds check for each flock		Positive samples of bird/flock	
Location	No.	%	No.	%
Baqubah	5	25	18	72.0
Al-Mokdadia	2	4	2	50.0
Kanan	4	16	12	75.0
Baladrose	4	16	10	62.5
Al-Kales	3	9	6	66.6
Al-Mansoria	2	4	2	50.0
Total	74		50	

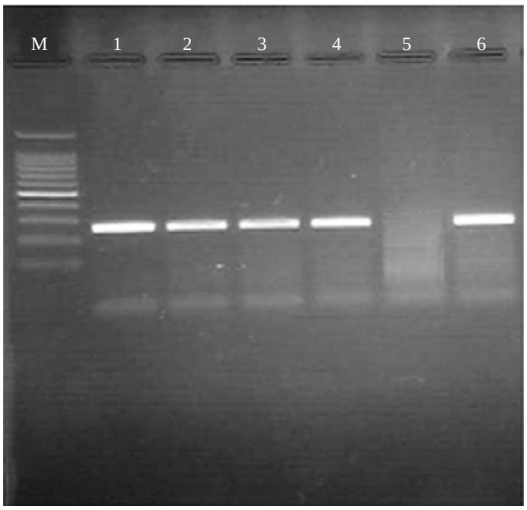

Fig. 1: RT-PCR result showing positive detection of IBV M-gene as revealed by positive bands following electrophoresis on 1.5 agarose gel. Lane M: 100 bp DNA marker, Lane 1, 2, 3, 4: Positive samples, Lane 5: Negative control (No band appears), Lane 6: Positive control with DNA band bp

RESULTS

Rapid immunochromatographic assay: In this study immunochromatographic assay revealed that 155 out of 200 (77.5%) fecal samples collected from 20 flocks were positive for IB as evidenced by the appearance of a purple band on both the test and control line. The result was expressed in percentage for all the farms (Table 2).

Reverse transcriptase polymerase chain reaction: Using the infectious bronchitis virus detection RT-PCR kit, 50 out of 74 IBV suspected samples 67.5% (50/74) were determined positive for IB as evidenced by the appearance of the expected DNA band with a size of 261 bp on agarose gel (Fig. 1).

The total number of RT-PCR positive samples detected in this study are summarized in Table 3.

DISCUSSION

Infectious Bronchitis (IB) is one of the most economically important viral diseases of poultry. It is prevalent in virtually all

countries with an intensive poultry industry[13,14]. Today, numerous IBV variants have been identified in different countries in the world[5]. In Iraq, IB has been reported in most of the regions. However, to provide an update on the epidemiology of the disease in Diyala province, the present study was conducted. The study, reported a high rate of IB seropositivity in the Diyala province, Iraq. Previous finding observed 20-40% mortality rate in flock of chickens showing clinical and gross pathological evidences of IB in Diyala[15]. This high seropositive rate observed in this study is similar to the report made by other researchers who conducted similar survey in other parts of Iraq[16,17]. The use of more powerful molecular assays have been reported to yield better accuracy due to their increased sensitivity and specificity, hence RT-PCR assay targeting a conserved M-gene of IBV was used to further gain insight on the molecular epidemiology of the disease. RT-PCR analysis revealed that 67.5% of the samples tested positive to IBV as evidence by amplified gene sizes relative to that of positive control. The disparities observed between, serological test and RT-PCR results is not unexpected since RT-PCR detect the presence of intact viral gene which likely inform about the presence of infectious virion[18]. On the other hand, chickens that seroconvert might show evidence of detectable antibodies without the necessary presence of viral antigen. Previous study reported similar detection rate of 42.8% in broiler flocks from different regions in the neighboring Iran[19]. Similarly, a study in Jordan reported similar RNA detection rate (58.8%) when examining 25 broiler flocks suffering from respiratory distress[20].

CONCLUSION

Both serological and molecular evidences of IB have been demonstrated in Diyala province of Iraq. Thus poultry rearing in this locality may be faced with health and production challenges due to sporadic outbreak of the disease. Hence there is the need for constant monitoring, vaccination exercise and biosecurity measures to reduce the menace of the disease. Future studies are needed to identify the local IBV variants circulating in this locality and to carry out antigenicity test as well as vaccine-match analysis to identify suitable vaccine for control and preventions.

SIGNIFICANCE STATEMENTS

This study discovered and reports high antibody and viral RNA detection rates due to infectious bronchitis virus in poultry in Diyala Iraq, unlike previous studies, our serological findings are consistent with previous results. Interestingly, these findings have been substantiated through the use of more sensitive RT-PCR results, which further serve as confirmation for the circulation of infectious bronchitis virus among poultry. The present study underscores possible involvement of infectious bronchitis virus in the sporadically reported outbreaks in poultry industry. These findings are important for poultry farmers as well as policy markers when designing vaccination strategies to curtail the economic losses due to IB, thus, boosting poultry production and ensuring protein supply to the general public.

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